EFFECT OF LONG-TERM FEEDING OF GRADED LEVELS OF DEOXYNIVALENOL TO GROWER-FINISHER PIGS

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By

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ABSTRACT

The prevalence of deoxynivalenol (DON) concerns swine producers in Western Canada. There has been extensive research into the effects of DON in pigs, much of which targets young animals and/or in short-term studies. The objectives of this thesis were to determine the effects of long-term exposure to DON-contaminated diets on growth performance, carcass characteristics, and health in finisher (Exp. 1) and grower-finisher (Exp. 2) pigs. In experiment 1, 200 pigs were housed in groups of 5 pigs/pen (n=10 pens/treatment) for a 6-wk feeding trial. In experiment 2, 240 pigs housed in groups of 6 pigs/pen (n=10 pens/treatment) were used in an 11-wk feeding trial. Pigs were fed a control diet without DON (CONT) or the basal diet with 1, 3, or 5 ppm DON (DON1, DON3, and DON5, respectively). Weekly pig BW and pen-wise feed intake was recorded to determine average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F). Blood samples were collected on d 0, 14, and 42 for Exp. 1 and d 0, 14, and 42 and on d 42, 56, and 84 for Exp. 2. Serum was analyzed for liver and kidney health and immune response to a vaccine challenge. Carcass data was collected at the end of Exp. 2. In both studies, nitrogen (N)-balance was conducted to determine the effect of DON on N-utilization. In Exp. 1, pigs fed DON3 and DON5 had consistently reduced ($P < 0.05$) ADFI and ADG from d 0-28 compared to CONT and DON1, after which there was no effect ($P > 0.05$) on ADFI, ADG, and G:F. N-retention was reduced ($P < 0.05$) in DON3 and DON5 pigs. In Exp. 2, DON3 and DON5 diets reduced ADG ($P < 0.05$) during the grower phase and the overall experimental period compared to CONT-fed pigs. There was no treatment effect on ADG in the finisher phase ($P > 0.05$) but ADFI during the first week was lower ($P < 0.05$) in DON3 and DON5-fed pigs compared to CONT and DON1. Compared to CONT, ADFI in the finisher phase and overall was lower ($P < 0.05$) in DON-fed pigs compared to CONT. For both phases, there was no DON effect ($P > 0.05$) on G:F. Finisher N-balance results showed no impact of DON intake on N-retention ($P > 0.05$), however, N-retention was reduced in the grower pigs fed DON3 and DON5 diets ($P < 0.05$) compared to CONT. There were no treatment effects ($P > 0.05$) on carcass traits, health, or immune response. The lack of effect on G:F suggests negative effects of DON on growth performance are largely due to impaired feed intake. Overall, the performance was less affected in the grower-finisher study relative to the finisher study. Further, regardless of age, there was evidence that pigs can adapt to DON over the long-term. This information will allow producers to adjust feeding programs to account for reduced performance due to dietary DON.

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DEDICATION

I dedicate this thesis to my parents Mr. Francis and Mrs. Vida Otchere Bosompem

Thank You.

"Captain of Israel's host, and guide
of all who seek the land above,
beneath your shadow, we abide,
the cloud of your protecting love.
our strength, your grace; our rule, your word:
our end, the glory of the Lord.

By thy unerring Spirit-led,
we shall not in the desert stray;
we shall not full direction need,
nor miss our providential way.
as far from danger as from fear
while love, almighty love, is near."

By
Charles Wesley

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LIST OF ABBREVIATIONS

ADFI	Average daily feed intake
ADG	Average daily gain
AgRP	Agouti-related protein
AIA	Acid insoluble ash
AOAC	Association of Official Analytical Chemists
AST	Aspartate aminotransferase
ATTD	Apparent total tract digestibility
BFT	Backfat thickness
BW	Body weight
℃	Degrees Celsius
CCAC	Canadian Council on Animal Care
CDS	Condensed distiller's solubles
CFIA	Canadian Food Inspection Agency
CGC	Canada Grain Council
CONT	Control
CCK	Cholecystokinin
CV	Coefficient of variation
d	Day(s)
DDGS	Distiller's dried grains with solubles
DM	Dry matter
DOM	Deepoxy-deoxynivalenol
DON	Deoxynivalenol
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation
FDK	*Fusarium* damaged kernel
FHB	*Fusarium* head blight
Fig	Figure
G:F	Gain to feed ratio
GGT	Gamma-glutamyl transferase

GGT	Gamma-glutamyl transferase
GLDH	Glutamate dehydrogenase
h	Hour(s)
H2SO4	Sulfuric acid
HPLC	High-performance liquid chromatography
IgG	Immunoglobin G
IU	International units
K	Potassium
kg	Kilogram
LD	Loin depth
ME	Metabolizable energy
MeOH	Methanol
mg	Magnesium
mg	Milligram
min	Minute
mL	Milli litre
mM	Milli molar
MS	Mass spectrometry
N	Nitrogen
NE	Net energy
nm	nano meter
NRC	National Research Council
PBS	Phosphate buffered saline
PD	Protein deposition
PNPP	P-nitrophenyl phosphate di(tris) salt crystalline
ppb	Parts per billion
ppm	Parts per million
PYY	Peptide YY
r^2	Multiple correlation of determination
rcf	Relative centrifugal force
rpm	Revolutions per minute

SAS	Statistical analysis software
SEM	Standard error of means
SID	Standard ileal digestible
SW	Slaughter weight
TBST	Tris-buffered saline containing 0.05% Tween 20
µL	Micro litre
µmol	Micro mole
UV	Ultraviolet
v/v	Volume/volume
ZEA	Zearalenone

Publication Disclosure

Some of the material/data presented in this thesis has been accepted for publication in the Journal of Animal Science as follows

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Author contribution

M. A. Bosompem conducted the animal work, collected the data, completed the lab analysis, and assisted with the statistical analysis and manuscript preparation.
M.O. Wellington assisted with data collection, lab analysis, conducted the statistical analysis, and wrote the manuscript
R. Petracek assisted with the conduct of the animal work and data collection.
V. Nagl assisted with the analysis of DON in feed and biological samples and manuscript review
D.A. Columbus conceived the study and designed the experiment, obtained funding for the work, supervised the work, and assisted in the manuscript writing.
All authors read and approved the final manuscript.

1.0 INTRODUCTION

Mycotoxins are a major contributor to grain contamination and have been reported to be increasing yearly (BIOMIN, 2019). For example, in a 2019 Biomin report, about 79% of wheat sampled in North America was contaminated with deoxynivalenol (DON), a *Fusarium* mycotoxin (Bushnell et al., 2003). It is estimated that in North America, about 5 billion dollars in agricultural revenue is lost due to mycotoxin contamination of cereal grains (Wouter et al., 2019). There is evidence suggesting that DON and other harmful mycotoxins increase in incidence annually (Bianchini et al., 2015), rendering large quantities of cereal grains unavailable for human food production, instead of being downgraded for use in livestock feed. Thus, there is a need to find practical ways of using these contaminated grains in animal production systems as a feed resource. The Canadian Feed Inspection Agency (CFIA) has suggested feeding limits for many mycotoxins for different livestock species. For example, in pigs, a maximum of 1 ppm DON is allowable in complete diets, irrespective of the age of the pig. Feeding DON-contaminated diets to pigs above the CFIA recommendations has been reported to have negative effects on growth performance and health of pigs (Trenholm et al., 1994; Girardet et al., 2011; Van Le Thanh et al., 2015). For example, when diets contaminated with DON above allowable limits were fed to weanling pigs, Weaver et al. (2013) reported and Van Le Thanh et al. (2015) reported reduced growth performance. However, the effects of DON intake have been inconsistent, with other studies reporting no effect of feeding DON-contaminated diets on pig health and growth performance (Øvernes et al., 1997; House et al., 2002). Indeed, differences in health and growth performance response of pigs fed DON-contaminated diets are thought to be related to the physiological age, the concentration of DON in the diets, and the duration of exposure (House et al., 2002; Øvernes et al., 1997). For example, it is suggested that younger pigs may respond more negatively relative to finishing pigs to DON, but the duration of exposure may also influence the response (Øvernes et al., 1997; House et al., 2002; Weaver et al., 2013; Van Le Thanh et al.,2015). Given the increasing incidence of DON contamination of grains over the last few years, there is the need and our responsibility to evaluate strategies to utilize DON-contaminated grains in feeding pigs, including examining the potential for adaptation and recovery from the DON-induced impact on health, and growth performance for agricultural sustainability. Therefore, the focus of this thesis was to determine the impact of long term feeding of DON-contaminated diets in growing-finishing pigs on health, nutrient utilization, growth performance, and carcass characteristics.

2.0 LITERATURE REVIEW

2.1 Introduction

Mycotoxins are secondary metabolites produced by molds and fungi which, when ingested, exert toxic effects resulting in adverse physiological responses in animals and humans (Peraica et al., 1999; Bonnet et al., 2012; Marroquín-Cardona et al., 2014). This contamination is the result of cereal grains that are infested with molds or fungi, which produce mycotoxins while in the field or under storage (Schuh 2013; Schatzmayr and Streit, 2013). About 400 mycotoxins have been identified, however, of principal importance to animal agriculture are aflatoxins (AF), T-2 toxin (T-2), HT-2 toxin (HT-2), DON, nivalenol (NIV), fumonisin (FUM), zearalenone (ZEA), ochratoxin A (OTA), and ergot alkaloids because they are commonly found in cereal grains (e.g. wheat, barley, and corn) used for livestock feed (Wu, 2004; Schuh, 2013). The incidence of mycotoxins is on the rise in recent years mainly due to the fungal adaptation to environmental changes attributed to global climatic variations (Canadian Grain Commission, 2019; FAO, 2018) as well as increased international trade and transport of agricultural goods (FAO, 2018). There is evidence suggesting that increasing global temperature and erratic weather conditions have increased the proliferation of new fungal species in locations where mycotoxin prevalence was historically low (Binder et al., 2007). For example, in North America, during the 2019 crop year, the incidence of DON contamination in grain samples had risen to 79 % from 65 % in 2018. Even more remarkable was the increase in ZEA contamination from 26 % in 2018 to 56 % in 2019 (Biomin, 2018; 2019). The Government of Saskatchewan, in its Economic Review, reported that approximately 12.90 and 14.41 million tonnes of wheat was harvested in the 2017 and 2018 cropping seasons, respectively (Saskatchewan, 2017; 2018). Contamination by *Fusarium* alone was reported to have caused about 2.1 million tonnes of wheat to be downgraded in 2018, representing 14.2% of the total wheat production (Canada Grains Council, 2020). With the reported effects of mycotoxins on animal health and growth performance, avoiding these contaminated grains would be the ideal approach, however, downgraded grains due to mycotoxin contamination are used in livestock feed, making the feeding of these grains difficult to avoid (Bianchini et al., 2015; Tittlemier et al., 2013b).

Since it is not entirely possible to avoid mycotoxin contamination of grains, the CFIA has set regulatory guidance limits for different mycotoxins in complete diets for livestock species. For example, cattle and poultry can be fed diets containing up to 5 ppm DON, while a maximum of 1

ppm is allowed in swine diets. However, with sustainable animal production in mind, it is to an extent our responsibility to determine methods to utilize feedstuffs that are not suitable for human consumption, including DON-contaminated grains. This review will highlight some reported effects of mycotoxin contamination (mainly DON) in swine diets and the effects on health, nutrient utilization, and growth performance. Further, this review will elucidate some possible mitigating strategies that allow for the utilization of DON-contaminated grains in swine diets.

2.2 Economic importance of mycotoxins in animal agriculture

The economic impact of mycotoxins and specifically DON, in animal production is yet to be fully explored and understood (Marroquín-Cardona et al., 2014). Recent articles in 'All About Feed' in 2016 discussed the issues of mycotoxins, stating that with advances in mycotoxin analysis it has become clear that the mycotoxin problem is much larger than once imagined and the costs associated with mycotoxin contamination will worsen with climate change, as has been supported by data from the FAO (2018). It has been reported that about 25% of all food produced globally is exposed to mycotoxin contamination. Further, global losses of food due to the prevalence of mycotoxins is about 1 billion tonnes annually (Iheshiulor et al., 2011). The exact extent of this problem is difficult to determine because analysis of mycotoxin levels in feed is highly variable and dependent on sampling techniques and the analytical methods used (Whitaker et al., 2000; Hallier et al., 2011). Total economic losses attributed to mycotoxins, as identified by Wu (2007), are made up of market rejection losses and animal production losses. Some of the costs associated with mycotoxins include laboratory analysis, quality management and regulations, research and development, agricultural extension, and legal issues, as well as reduced animal performance and health and downgrading of grains for human consumption to use in livestock feed (Charmley et al., 1995; Marroquín-Cardona et al., 2014). Others include medical costs, management strategies to reduce exposure to mycotoxins, and import restrictions on animal feed ingredients (Marroquín-Cardona et al., 2014). Another major cost that can be attributed to mycotoxins is the recall of animal products, such as milk and eggs from the dairy and poultry industries, respectively (Sobrova et al., 2010; Winstanley, 2013).

With Canada as the 3rd largest global exporter of wheat behind the USA and Russia according to FAO 2017 data and the 10th largest pork producer, with approximately 2.1 million tonnes, it is clear how important these industries are to Canada and Saskatchewan's economy. The

constant annual increase in production of wheat, oats, barley, and other grains has, unfortunately, been supporting the proliferation and adaptation of *Fusarium* due to the relative increase humidity of recent seasons compared to former seasons, the availability of substrate for reproduction, and the movement of contaminated grain across the country. This, therefore, results in a long-term challenge to grain producers in Saskatchewan and Canada (Saskatchewan, 2018; Canada Grains Council, 2020). Further, the cost of mycotoxin management may keep increasing unless dependable, affordable, and practical methods of controlling mycotoxins are developed and made readily accessible. With a quarter of the world's food crops affected by mycotoxins and thus, the real and apparent economic impacts call for the need to be able to adequately quantify these losses (CAST, 1989; Robens and Cardwell, 2003; Iheshiulor et al., 2011), for example, improving the variability and inconsistency of testing (Whitaker et al., 2000; Marroquín-Cardona et al., 2014).

2.3 Deoxynivalenol (DON) in animal agriculture

Deoxynivalenol is significant to agriculture because it is s the most prevalent mycotoxin affecting cereal grains (Rotter et al., 1996; Chaytor et al., 2011). The World Mycotoxin Survey (BIOMIN, 2019) stated that DON occurrence in many grain samples tested in North America, such as corn, barley, wheat, and soybean, had risen from 67% in 2018 to 79% in 2019. While all farm animal species are affected to some extent by DON contamination (D'Mello et al., 1999; Placinta et al., 1999), physiological and genetic differences in species result in varied responses to DON exposure (Santurio, 2000; Bonnet et al., 2012). The common response parameter observed with DON contamination among different species is poor feed intake, which leads to impaired growth and poor health (Gajęcki, 2010). The pig is particularly susceptible to DON contamination, with cattle and poultry showing reduced susceptibility and severity of response (Savard et al., 2015).

The in vivo toxicokinetics of DON has been studied and reported on extensively in the literature (Rotter et al., 1996; Seeling et al., 2006; Pestka, 2007; Nagl et al., 2012; Schwartz-Zimmermann et al., 2017), and even though the efficacy of intestinal uptake and metabolism of DON varies across farm animal species, all species possess some level or capacity to detoxify DON in the body (Maresca, 2013). It is, however, the extent of the detoxification and excretion that makes DON more or less toxic in one animal species versus another. Also, having the capacity to detoxify DON to its less toxic forms, for example, DOM-1, in the upper gastrointestinal tract before the small intestine also influences the susceptibility of a species to DON (Rotter et al., 1996;

Pestka, 2007; Maresca 2013). Anaerobic bacterial de-epoxidation and the conjugation of DON to glucuronic acid in vivo are reported to be the two important metabolic pathways of DON detoxification (Nagl et al., 2012). In mammals such as the pig, glucuronidation is the key metabolic pathway of detoxifying DON. This process enhances the polarity of the toxin increasing the excretion in urine and feces (Nagl et al., 2012).

In ruminants, while the classic responses of feed refusal and impaired feeding behavior are observed when feeding diets containing DON (D'Mello et al., 1999), there is evidence of biotransformation of DON in the rumen due to the large microbial population (Upadhaya et al., 2010), which is likely one of the reasons for the reduced impact of DON. In calves and lactating cows, susceptibility is higher compared to older cattle (Seeling et al., 2006). It has been reported that when DON-contaminated diets are fed to lactating cows an acute immune response can result (Marczuk et al., 2012). Even though ruminants are known to have the ability to bio-transform DON, lactating cows also have been reported to directly pass on DON and other mycotoxins, such as AF, to newborn calves and humans through milk and milk products (Sobrova et al., 2010; Astley, 2013; Winstanley, 2013). Various studies have shown varied results of DON on ruminants. For example, while some studies suggest no impact of DON on lactating cows fed DON up to 104 mg cow/day (Trenholm et al., 1984; Charmley et al., 1993; Ingalls, 1996) others reported reduced feed intake (Trenholm et al., 1984), reduced rumen microbial protein, and increased rumen ammonia nitrogen (Dänicke et al., 2005). Although reports have shown varied effects of DON on ruminants, largely at relatively lower levels, DON tends to be easily controlled and broken down by rumen enzymes, micro-flora, and fauna such as bacteria, fungi, and protozoa, and so, in general, there is little to no negative effect on ruminants. For example, the enzyme de-epoxidase has been reported to detoxify DON (Upadhaya et al., 2010; Gallo et al., 2015). Rumen fluid has been shown to change pure DON into less toxic forms, de-epoxy-deoxynivalenol (DOM-1) (Kollarczik et al., 1994) but no DON degrading strain could be cultured (Schuh, 2013) until the BBSH 797 strain of bacteria was isolated in 2006 and has now become the active ingredient in some commercial products used in Europe (Fuchs et al., 2002; Plank et al., 2009; Awad et al., 2010; Sayyari et al., 2018). However, at significantly higher concentrations, and in the presence of other mycotoxins, DON can cause rumen dysfunction. When the rumen function is impaired, DON reaches other parts of the ruminant gut, leading to responses such as immunosuppression, low feed intake, and impaired growth (Gajęcki, 2010). The economic impact of DON on cattle is reported to be higher

in dairy cattle due to their incredibly high-performance levels compared to beef cattle (Seeling et al., 2006).

In poultry, it is reported that response to DON is often observed at higher levels than that known for swine (e.g. 5 ppm; Trenholm et al., 1984) and that this level of intake has been associated with the increased rate of passage of digested feed from the intake to excretion, leading to significantly less time for DON to remain in the gut, reducing bioavailability (Prelusky et al., 1986). In poultry, DON reduces feed intake (Swamy et al., 2014), increases oxidative stress (Ghareeb et al., 2015), and impairs nutrient uptake (Awad et al., 2010). In turkeys, immunosuppressive effects of DON ingestion lead to increased susceptibility to disease (Chowdhury et al., 2005a; 2005b). As with ruminants, chickens have a mechanism of microbial detoxification of DON in the gut to the less toxic DOM-1. It has also been reported that poultry products such as eggs contain traces of DON (Bhat et al., 2010), which may lead to further losses due to product recalls.

Animal models, such as rodents, have been used to study and understand DON and its impact on animal systems. Arnold et al. (1986) used mice and rats to understand the mechanisms by which DON affects animals and concluded that DON acts as a gastro-intestinal irritant and impairs the function of the immune system (Pestka et al., 1987). In mice, DON ingestion reduced growth performance, and this was attributed to reduced feed intake. It was further explained that the lack of appetite was due to the regulation and deregulation of satiety hormones, such as peptide YY (PYY) and cholecystokinin (CCK), by DON and DON metabolites in circulation (Challis et al., 2003; Flannery et al., 2012).

2.4 Effect of deoxynivalenol (DON) on swine production

2.4.1 Effects of feeding DON-contaminated diets on growth performance and feed efficiency

Previous research indicates that DON adversely affects the overall growth performance of pigs regardless of the length of exposure. For example, Kong et al. (2015) fed 14.6 mg DON per kg of feed to growing pigs and saw about a 70% drop in the average daily gain (ADG) when compared to pigs fed uncontaminated diets. Similarly, Serviento et al. (2018) reported that when pigs were fed DON-contaminated diets intermittently over a 28-d period, the overall ADG was lower in the DON-fed pigs compared to pigs fed DON-free diets. Reduction in growth performance of DON-fed pigs is suggested to be because of the effect of DON effect on feed intake. Impaired

feed intake is the main response of pigs to DON ingestion, and as dietary DON concentration increases, vomiting and complete feed refusal occur (Morrissey and Vesonder, 1985; Rotter et al., 1996; Kong et al., 2015; Serviento et al., 2018). These effects lead to lower average daily feed intake (ADFI) and reduced growth and feed efficiency (Kong et al., 2015; Serviento et al., 2018). In pigs, age, previous exposure, and duration of exposure to DON have been shown to affect the overall impact of DON on feed intake (Serviento et al., 2018; Nguyen-Ba et al., 2020), which largely agrees with results from Flannery et al. (2011).

In other studies, results have suggested little to no impact of DON ingestion on pig growth performance. For instance, House et al. (2002) observed no effect of 1 and 2 ppm dietary DON intake on ADG and feed efficiency but saw a significant reduction in ADFI. Likewise, Dänicke et al. (2004) reported no effect on growth performance (ADFI, ADG, and feed efficiency) in starter and grower pigs fed DON-contaminated diets (3.86 mg/kg feed) over a 35-d period. Others have reported interesting results such as a significant reduction in ADG by DON intake whereas ADFI and feed efficiency were unaffected (Li et al., 2018). Further, Reddy et al. (2018) saw no negative effect of DON on ADFI, but ADG and feed efficiency were significantly reduced, which led to an overall reduction in final BW. While the inconsistencies in the response to dietary DON ingestion in response parameters such as ADFI, ADG, and feed efficiency may be due to differences in dietary DON level, age of pig, and physiological state, this may also be evidence of variability in individual animal response (Kong et al., 2015; Serviento et al., 2018). The impact of DON on pigs may also be influenced by pig gender. It has been proposed that gilts are more susceptible and show greater negative responses compared to barrows (House et al., 2002). Cote et al. (1985) conversely reported that males respond more severely to DON contamination.

While the initial acute response to DON is well-characterized, there is evidence suggesting that when pigs are exposed to DON-contaminated diets for longer periods, there is the potential for reduced adverse response or adaptation over time. For example, Serviento et al. (2018) observed a 53% drop in ADG in pigs after initial exposure to DON**.** When DON was withdrawn and reintroduced, DON-fed pigs showed a 39% drop in ADG compared to control pigs. This suggests that subsequent exposure to DON will result in a reduced negative effect (Serviento et al., 2018) though, in this experiment, the pigs were older during the second exposure. It was also reported by Serviento et al. (2018) that after the immediate reduction in ADFI in pigs exposed to DON, intake and ADG recovered over 7 d to levels similar to those reported before DON exposure,

suggesting that pig performance can recover. In another study, Sayyari et al. (2018) fed pigs with diets up to 5.7 ppm DON and found no difference in ADFI and ADG after 5 weeks compared to pigs fed diets with no DON. Multiple or repeated exposures to DON at higher intake levels are thought to afford an improvement in the ability to tolerate lower doses (Flannery et al., 2011). Furthermore, an exposure of about 28 d has been recommended for a total recovery and adaptation to diets contaminated with DON, hence the reason why relatively shorter exposures to DON lead to more severe impacts (Serviento et al., 2018).

2.4.2 Effects of feeding DON-contaminated diet on health and immune response

Fusarium mycotoxins have potential immunomodulatory effects, with the immune system often considered the second most sensitive physiological system to DON toxicity after the gut (Maresca, 2013). For example, in the review by Maresca (2013), it was reported that DON and its derivatives can negatively impact immune cells such as B- and T-lymphocytes, natural killer cells, and macrophages in a dose-dependent manner. Increased blood cell counts (monocyte and hematocrit) in pigs fed DON-contaminated diets have been reported (Pinton et al., 2008; Chaytor et al., 2010). Other studies have reported an increase in serum antibodies, IgA and IgM, when pigs were fed DON-contaminated diets (Swamy et al., 2002; Goyarts et al., 2005; Tiemann et al., 2006). Pro-inflammatory cytokines such as TNFα, play a regulatory role in immune response function (Wu, 2018). There is evidence that when pigs are fed diets contamination with both DON and AF, there is an alteration in the concentration of TNFα. Attempts to characterize the negative effects of DON on the health of pigs have produced conflicting results, as some studies have reported that DON contamination of up to about 5 ppm does not affect blood chemistry parameters such as total proteins, enzyme activity, and phosphorus (Chaytor et al., 2011; Trenholm et al., 1994). Similarly, Rotter et al. (1995) reported only a temporary alteration in serum proteins in blood samples of DON-fed piglets and Accensi et al. (2006) reported that DON (840 µg/kg feed) fed to piglets did not affect immunoglobulin, lymphocyte, and cytokines concentration.

2.4.3 Effects of DON-contaminated diets on organ and intestinal health and function in pigs

The effect of DON contamination in pig diets has been reported to cause pathological changes and damage in different organs including the gut, liver, kidney, and spleen (Grenier et al., 2013; Schuh, 2013). Further, organ damage such as liver fibrosis, bile duct hyperplasia, and

megakaryocytosis was reported in pigs fed diets contaminated with DON (Harvey et al., 1991; Chaytor et al., 2011; Grenier et al., 2013). There is evidence that suggests that feeding animals DON-contaminated diets has a negative effect on the overall health of the gut. Studies conducted both in vitro and in vivo have demonstrated that DON may inhibit the absorptive function and intestinal barrier permeability (Maresca et al., 2002; Grenier et al., 2013). In weanling pigs, reduced expression of tight junction proteins (e.g. occludin, claudin, etc.) was observed in the ileum of pigs fed DON-contaminated diets (Lessard et al., 2015). Indeed, evidence suggests that DON influences intestinal health and integrity, altering mucosal immune defense and antioxidant systems in the gut (Pinton et al., 2008; Accensi et al., 2006; Bracarense et al., 2012). The stability of the intestinal microbiome is an important parameter for intestinal health; therefore, the effect of DON contamination of intestinal health may be related to the effect of DON on the intestinal microbiome. Waché et al. (2009) studied the impact of feeding a DON-contaminated diet on the intestinal microbiome in pigs and concluded that DON led to an imbalance of the dynamics of the intestinal microbial communities. Overall, there is evidence on the impact of DON on intestinal health and function, as such mitigation strategies for DON, should focus on mitigating the impact on the gut.

2.4.4 Effects of feeding DON-contaminated diet on carcass characteristics in pigs

The impact of DON on carcass characteristics (carcass weight, backfat, and lean depth) has been reported in barrows and gilts and data suggests that DON has no negative effects (House et al., 2002). Likewise, Bergsjø et al. (1993) reported that while there was reduced carcass weight (65.3 kg vs 76.7 kg) and increased liver size (2.53% vs 2.25% of body weight) in pigs fed DON-contaminated diets compared to DON-free diets, dressing percentage and lean yield were not different.

2.4.5 Effects of feeding DON-contaminated diets on other physiological functions

Some physiological functions have been reported to be affected by DON, largely these effects impact feeding behavior and feed intake (Flanery et al., 2012; Maresca 2013). For example, DON has been reported to affect neuroendocrine signaling, such as those related to serotonin, which can affect anorexigenic (e.g. insulin and leptin) or orexigenic (e.g. ghrelin) hormones, which can alter feed intake (Watterson et al., 2013; Pinton and Oswald, 2014). In mice, Flannery et al.

(2012) reported that DON exposure increased plasma levels of two key appetite-regulating hormones - peptide YY and cholecystokinin. Also, subcutaneously administered purified DON in male rats reportedly leads to poor feed intake and hormonal and metabolic dysfunction (Szkudelska et al., 2002). Additionally, hormones such as leptin and insulin, which are responsible for energy balance and the metabolism of carbohydrates, fats, and proteins, have been reported to be reduced in mice when fed 10 mg/kg DON (Kobayashi-Hattori et al., 2018). Even though the models used for these experiments were rodents, swine share similarities with these species in terms of metabolism.

2.5 Determination of DON contamination

A necessary step in mitigating the effects of DON in swine production is accurately determining DON content in feed ingredients and complete feeds. Unfortunately, there are large discrepancies and variability when it comes to the accuracy of testing for DON (Whitaker et al., 2000; Beaulieu et al., 2009). For example, DON tested samples from the same source, sampled at the same time, but from different parts of a containing vessel can affect the overall accuracy of test results. For example, Whitaker et al. (2000) measured DON in 32 wheat samples from 24 lots and showed significant variations. Further, there is evidence that grain dust contains higher concentrations of DON than actual grains from the same sample (Sanders et al., 2013). For better consistency, less variability, and higher confidence in test results, Champeil et al. (2004) suggests the use of ground grain samples instead of whole-grain-based lab testing. Additionally, harvesting and storing procedures were shown to have an effect on DON levels in tested samples (Champeil et al., 2004). Hallier et al. (2011) added that during testing for DON, a longer duration of extraction showed significantly higher concentrations of DON compared to shorter duration, sample size had no effect on results but grinding or flour particulate size significantly affected the concentrations of DON recorded. With these challenges in mind, it is worth considering the biological analysis of samples such as blood and urine since extensive works by Goyarts and Dänicke, (2005), Nagl et al. (2012), and Schwartz-Zimmermann et al. (2014; 2017) have shown that there is a strong positive correlation between the intake of DON and its presence in the body of an animal. For example, when DON was introduced into mice, the level of DON in the bloodstream increased significantly and gradually reduced over time, within a few hours, there was practically no DON in the blood. This also supports the fact that, when DON is introduced into an animal, especially

monogastric species such as swine and poultry, it quickly undergoes breakdown by the liver into the less toxic glucuronide, while at the same time the kidneys actively expel the mycotoxin into the urine (Nagl et al., 2014), with the main route of excretion of absorbed DON in animals being via urine (Nagl et al., 2012). The specific effects of DON in any species are dependent on the level of DON present as well as the presence of other mycotoxins within the diet, with some mycotoxins having a synergistic effect when present together (Schuh, 2013).

2.6 Mitigation strategies for DON in swine feed ingredients and complete diets

While the best strategy to eliminate the negative effects of DON is to avoid feeding DON-contaminated feed to swine, the increasing incidence of DON and other mycotoxins in feedstuffs (Placinta et al., 1999; Zhang et al., 2018) has made this increasingly difficult (House et al., 2002; Schatzmayr and Streit, 2013; FAO, 2017). In recent times, the use of by-products from the food and biofuel industries, such as distiller's dried grains with solubles (DDGS), wheat middling, wheat bran, corn (maize) meal, hulls, and condensed distiller's solubles (CDS), in swine nutrition has become popular (Zijlstra and Beltranena, 2013; Woyengo et al., 2014). Unfortunately, these residual products end up containing concentrated levels of DON and other mycotoxins because the processing techniques are unable to deactivate or remove the toxin (Schaafsma et al., 2009). These challenges have also led to the use of techniques including blending contaminated ingredients with ingredients not contaminated with DON and other *Fusarium* mycotoxins which allows for 1) making efficient use of downgraded grains and highly DON concentrated co-products (Schaafsma et al., 2009); 2) maintaining profitable production by formulating diets to meet the regulatory requirements set by the CFIA (CFIA, 2017; Charmley et al., 1995); and 3) reducing the relative cost of feeding DON-contaminated diets as opposed to clean diets (Woyengo et al., 2014). The increasing threat of DON has resulted in substantial research investigating effective measures for removal or decontamination of DON in swine feed ingredients and feeds and/or mitigating the negative effects of DON ingestion. Generally, DON mitigating strategies can be classified into physical, chemical, or biological modes of action (Charmley et al., 1995; Zhu et al., 2016; Luo et al., 2018). Some of the more common strategies examined will be discussed below.

2.6.1 Physical methods of mitigating DON in feed ingredients and complete diets

Several physical methods of DON removal have been examined. For example, the use of physical abrasion to remove portions of the grain surface in a process known as pearling. In a study by Sovrani et al. (2012), DON content in grain was shown to be reduced by as much as 64% when the top 10% of the grain was pearled. This was generally in agreement with results from House et al. (2003), where 15 seconds of pearling resulted in the grains containing 34% of the initial DON analyzed (a 66% drop). The efficiency of pearling can be as high as 78% (Pinotti et al., 2016) but the process of pearling, though effective, also reduces the overall mass, crude protein, and fiber contents of the grains (House et al., 2003). Also, because pearling does not decontaminate DON, the mycotoxin may be redistributed in processing facilities through grain dust, which is known to contain significantly higher levels of DON compared to whole grains (Sanders et al., 2013). Another method is the use of near-infrared transmittance, applied by devices like the BoMill AB TriQ™ seed sorter, to sort *Fusarium* damaged kernels from bulk grain (Shahin and Symons, 2011; Kautzman et al., 2015). Other physical methods include quick-drying, the use of ultraviolet (UV) light, and the use of adsorption agents like clays (Zhu et al., 2016). Adsorbents are materials that have the binding potential to hold onto molecules on their surfaces (Shehata et al., 2000). The use of adsorbents, or binders as they are popularly known, relies on the fact that the physical structure of the binding agent gives it a charge coefficient that can form an attraction with a target molecule, in this case, DON (Kong et al., 2014). For example, some clays and dried yeast have shown some promise (Weaver et al., 2013).

Zhu et al. (2016) further reported that while adsorbents are effective against AF, they are generally ineffective against DON and other mycotoxins such as OTA, T-2, HT-2, etc. These adsorbents can, however, be modified with quaternary long-chain alkyl/aryl amine (NR_4^+) polymers which increases the hydrophobicity and adsorption potential, which may increase efficacy. On the contrary, some other studies have shown the efficacy of some other mycotoxin adsorbing agents in reducing the negative effects of DON on growing pigs (Awad et al., 2010). These inconsistent results have led to the modification of regular adsorbents with the other products yeast cell wall, lactic acid bacteria, and conidia of *Aspergilli*. Kong et al. (2014) reported in an in vitro study that yeast cell wall as a DON sequestering agent was the most potent among a group of adsorbents including 4 bentonite clays, cellulose A, Cellulose B, and activated charcoal. The yeast cell wall was able to bind about 22.9% of the DON in the sample followed by cellulose

B with 16.8 % and activated charcoal with 14.4%. All other agents recorded binding agents tested recorded efficacy of under 10% (range: 1.0 – 6.7%). Another challenge with these binders is that they are mostly non-selective and, in many instances, other nutrients such as vitamins, amino acids, and minerals are bound along with the mycotoxins. A suitable adsorbent material should be able to bind a wide range of toxins, have high adsorption potential, reduce the non-specific attraction for nutrient binding, and have properties similar to feed ingredients (Zhu et al., 2016).

2.6.2 Chemical methods of mitigating DON in feed ingredients and complete diets

The chemical methods of DON mitigation involve the use of chemical agents such as bases, acids, oxidizing agents, aldehydes, or bisulfite gases to alter the chemical structure or bioavailability of the toxin in the gut of the pig (Zhu et al., 2016). The chemical methods also included using gamma rays in a radioactive treatment which has been reported to be an efficient control for mycotoxins such as AF (Bhat et al., 2010). The use of gamma rays, however, does not seem to be feasible according to O'Neill et al. (1993) due to the high levels of irradiation required to destroy the toxin. Zinc compounds (Savi et al., 2015) and ozonation (Chaytor et al., 2011; Savi et al., 2015) are effective against DON decontamination. The main limitation to the chemical means of controlling DON in diets arises from their general high specificity for DON. This makes it easy for DON to avoid decontamination due to masking by proteins and carbohydrate molecules (Berthiller et al., 2005; Warth et al., 2013) and could result in an unexpectedly high free-DON content even after applying these methods. Other limitations include the increased overall cost of acquiring and application of these products and their use in pork production. Further, the required regulatory requirements and consumer acceptance may pose a challenge to their implementation.

2.6.3 Biological methods of mitigating DON in feed ingredients and complete diets

Biological methods for controlling DON, which includes the use of enzymes and microorganisms, such as bacteria and yeast (Dalié et al., 2010; Pizzolitto et al., 2012), seem to be the most desirable means of controlling DON, due to the high efficiency and specificity and low environmental impact (Zhu et al., 2016) of these methods. There are two main modes of action through which these biological methods reduce mycotoxins (Leibetseder, 2006). The first is the detoxification of mycotoxins and the prevention of the formation of secondary toxins (Leibetseder, 2006). The use of intestinal bacteria from poultry and ruminants has shown some promise with

converting DON to its less toxic form DOM-1 (Leibetseder, 2006). Leibetseder (2006) further reported in a review that there was a 55% reduction in DON concentration when 5 mg/kg DON-contaminated corn was treated with poultry digestive tract microbial inoculum, which also lessened the toxic effects on pigs. According to Awad et al. (2010), the effectiveness and economics will determine the practicality of using microorganisms for the catabolism and detoxification of DON in feed ingredients and feeds. In addition to the methods mentioned, other innovative methods include the use of essential oils (Luo et al., 2018), magnetic materials, and nano-particles (Chauhan et al., 2016; Luo et al., 2018).

2.6.4 Combining mitigation strategies of DON in conventional feed additives

A more recent strategy being examined to control the negative effects of mycotoxins is the use of feed additives which contain a blend of components, such as enzymes, probiotics, amino acids, antioxidants, adsorbents, aimed at detoxifying and binding mycotoxins in the feed as well as supporting animal health. For example, Van Le Thanh et al. (2015) reported that when four feed additives were tested for efficacy, diets containing preservation components showed promise of restoring the performance of pigs, whereas contaminated diets containing aluminosilicate (silicate clay), glucomannan (dietary fiber), yeast, live bacteria, enzymes, and plant extracts individually had no effect. The use of a commercially available preservative blend was also reported to be effective against DON in contaminated diets (Patience et al., 2014). While no feed additives have been approved in North America, specifically the U.S and Canada, for the control of DON in contaminated diets, there are some significant successes reported in Europe. Plank et al. (2009) reported that two products, Biomin® BBSH 797 and Mycofix Plus® 3.E, approved by the European Food Safety Authority were indeed capable of reducing the negative effects of DON on growing pigs but Sayyari et al. (2018) showed no effect when an additive with the bacterial strain *Coriobacteriaceum* DSM 11798 (the active component of Biomin® BBSH 797) was used in growing pigs. Other commercially available products include Defusion (Akey, Inc., Lewisburg, OH) or Integral (Alltech, Nicholasville, KY). With these varying results and a scarcity of information regarding the effectiveness of various mycotoxin products available commercially (Patience et al., 2014), further research on the development and use of feed additives to mitigation mycotoxins, and specifically DON, is required.

In conclusion, the above review has outlined and established the reported effects of DON-contaminated diets of swine and are now fairly known but over the short term mostly. The review also attempted to highlight some of the challenges surrounding DON contamination both in the grain and swine production sectors, as well as potential control and mitigation approaches available. However, when the increasing incidence of DON contamination is considered, there is an obvious economic impact of mycotoxin contamination for both the grain and pork sectors. Besides, with the lack of effective mitigation strategies, specifically for DON-contamination in diets for pigs in Canada, further information is required on more practical and feasible strategies such as the long-term DON exposure in grower-finisher pigs. Since production systems largely follow a long-term feeding regime, this information can be used to develop feeding programs that maximize the inclusion of DON-contaminated grains while minimizing the impact on growth performance and profitability of both pork and grain producers.

2.7 Objectives and hypotheses

The overall objective of this thesis was to determine the effects of long-term feeding of graded levels of DON in grower-finisher and finisher pigs.

The specific objectives of the studies were to determine:

1. The effects of DON levels on growth performance, nutrient utilization, and carcass characteristics.
2. The effects of DON levels on the overall health status of pigs.
3. The effects of DON levels on older pigs versus younger pigs.
4. The effects of dietary DON on DON levels in biological samples from pigs fed DON.

2.8 Research hypotheses

1. Increasing DON intake will initially reduce performance and nutrient utilization, which will recover over time. There will, however, be no negative effects of DON intake on carcass characteristics.
2. Increasing DON intake will result in negative effects on physiological indicators of health and immune function.
3. Older pigs will not experience severe effects in response to increasing DON intake and will require a shorter adaptation period compared to younger pigs.
4. The content of DON in biological samples will be correlated to intake levels of DON.

3.0 MATERIALS AND METHODS

The experimental protocols used in the present studies were reviewed and approved by the Animal Research Ethics Board of the University of Saskatchewan (AUP20130054) and followed the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

3.1 Animals, housing, diets, and experimental design

3.1.1 Experiment 1 (finisher pigs)

A total of 200 mixed-sex finishing pigs (Camborough Plus × C337; PIC., Canada) with initial body weight (BW) of 76.6 ± 3.9 kg were used in a 42-d experiment at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). The pigs were grouped-housed in pens (5 pigs/pen) in environmentally controlled rooms. The pens were randomly assigned to 1 of 4 dietary treatments (n=10 pens/treatment; Table 1). Dietary treatments consisted of a control diet (CONT) containing no DON or a diet containing 1, 3, or 5 ppm DON (DON1, DON3, or DON5). The basal diet was wheat-barley-soybean meal-based and formulated to be isonitrogenous and isoenergetic with nutrients meeting or exceeding the recommended requirement for finisher pigs (NRC, 2012). The dietary DON levels were achieved by replacing DON-free wheat with DON-contaminated wheat and wheat screenings proportionally according to the target DON levels. The mycotoxin profile and content of the DON-contaminated wheat and wheat screenings used in diet formulation were determined at Central Testing Laboratory (Winnipeg, MB, Canada). The complete experimental diets were analyzed for mycotoxins at Biomin Holding GmbH (Erber Campus 1, 3131 Getzersdorf, Austria; Table 2). The pigs had *ad libitum* access to feed and water. At the start of the growth performance trial, 1 barrow in each pen representing the average pen BW was selected for blood sampling and nitrogen (N)-balance. On the mornings of d 0, 14, and 42, blood samples were collected from the representative pig in each pen via jugular puncture while restrained with a hog holder. Blood samples were collected into heparin-coated and additive-free tubes (5 mL; BD vacutainer, BD, Mississauga, ON, Canada), which were temporarily stored on ice and then centrifuged at 2500 × *g* for 15 min to harvest plasma and serum, respectively. The plasma and serum samples were stored at -20 ℃ until further analysis. Serum samples were analyzed for DON and DON metabolite concentrations and markers of liver and kidney health and function.

Table 1 Composition of the diets used in experiment 1 (as-fed basis)[1]

Ingredient, %	CONT[2]	DON1[3]	DON3[4]	DON5[5]
Clean wheat	40.0	33.3	20.0	6.7
DON wheat [6]	0.0	4.9	14.8	24.7
Wheat screenings[7]	0.0	1.7	5.2	8.6
Barley	44.0	44.0	44.0	44.0
Canola oil	3.5	3.5	3.5	3.5
Soybean meal	10.0	10.0	10.0	10.0
L-Lysine-HCl	0.30	0.30	0.30	0.30
DL-Methionine	0.07	0.07	0.07	0.07
L-Threonine	0.10	0.10	0.10	0.10
Limestone	0.8	0.8	0.8	0.8
Dicalcium phosphate	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Vitamin/Mineral Premix[8]	0.2	0.2	0.2	0.2
Calculated Nutrient Content				
ME, kcal/kg	3282	3282	3282	3282
NE, kcal/kg	2502	2502	2502	2502
Dry Matter, %	86.5	86.5	86.5	86.6
Crude Protein, %	15.9	15.9	15.9	16.0
Lysine, % SID[9]	0.76	0.76	0.76	0.76
Calcium, %	0.50	0.50	0.50	0.50
Phosphorus, %	0.48	0.48	0.48	0.48
Analyzed Nutrient Content				
Growth performance diet				
Dry Matter, %	88.9	88.2	88.3	88.8
Crude Protein, %	14.6	14.2	13.5	14.8
DON[10], ppm[11]	0.11	1.34	3.58	5.72
Nitrogen Balance diet				
Dry Matter, %	88.5	88.1	88.9	88.7
Crude Protein, %	14.5	14.6	14.1	14.7
DON, ppm	1.56	1.32	3.09	4.94

[1]Nutrient content of diets based on the nutrient content of feed ingredients according to NRC (2012).
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]DON wheat contains 6.9 ppm DON (Central Testing Laboratory, Winnipeg Manitoba).
[7]Wheat screenings contain 32.8 ppm DON (Central Testing Laboratory, Winnipeg Manitoba).
[8]Supplied per kg of complete diet; vitamin A, 8000 IU; vitamin D, 1500 IU; vitamin E, 30 IU; menadione, 2.5 mg; vitamin B12, 0.025 mg; thiamine, 1.00 mg; biotin, 0.10 mg; niacin, 20 mg; riboflavin, 4 mg; pantothenate; 12 mg; folic acid, 0.50 mg; pyridoxine, 2.0 mg; Fe, 100 mg; Zn, 100 mg; Mg, 40 mg; Cu, 15 mg; Se, 0.30 mg; and I, 1mg.
[9]SID, standardized ileal digestibility
[10]DON, deoxynivalenol content analyzed by BIOMIN
[11]ppm, parts per million.

On d 35 of the experiment 1 pig/pen (identified at the start of the experiment) representing the average BW of the pen were individually housed in metabolism crates (56" × 58.5") in a temperature-controlled room (21 ± 2 ℃) for N-balance collection. The selected pigs from the growth performance trial remained on the same dietary treatment during the N-balance period. During the N balance period, the same basal wheat-barley-soybean- diets were fed except for the inclusion of celite (0.4%) at the expense of uncontaminated wheat. The daily feed allocation was set at 2.8 × maintenance metabolizable energy requirement (197 kcal/kg $BW^{0.60}$/d; NRC, 2012) and fed in two equal meals at 0700 h and 1500 h. Following a 7-d dietary and environmental adaptation period, total daily urine output and fresh-fecal grab samples were collected over a 2-d period. On the last day of the 2-d collection period, approximately 3-4 h post-prandial, blood samples were taken from pigs for serum DON analysis.

3.1.2 Experiment 2 (grower-finisher pigs)

A total of 240 grower-finisher pigs (Camborough Plus × C337; PIC., Canada) with an initial body weight of 35.9 ± 1.1 kg were used in a 77-d experiment at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). The pigs were group-housed in pens (6 pigs/pen) in environmentally controlled rooms (21 ± 2℃). The pens were randomly assigned to 1 of 4 dietary treatments (n=10/treatment; Table 2). Dietary treatments consisted of a control diet (CONT) containing no DON or a diet containing 1, 3, or 5 ppm DON (DON1, DON3, or DON5). Dietary DON content was achieved by replacing uncontaminated wheat with a proportional amount of DON-contaminated wheat and wheat screenings to attain the targeted DON levels in the final diet. The mycotoxin content of wheat and wheat screenings used in formulating the experimental diets were analyzed using ELISA at Central Testing Laboratory (Winnipeg, MB, Canada). The basal diet was wheat-barley-soybean meal-based and formulated to be isonitrogenous and isoenergetic with nutrients meeting or exceeding the recommended requirement for grower (25-75 kg) and finisher pigs (75-120 kg) according to the NRC (2012). The diets were fed according to a 2-phase protocol, with grower phase diets fed from d 0 – 42 and finisher phase diets fed from d 43 – 77. The pigs had *ad libitum* access to feed and water. At the beginning of the experiment, 2 pigs/pen were identified as representative pigs for the grower and finisher phases. On d 14 and d 56 of the experiment, the previously selected pigs in each pen were inoculated intramuscularly with a commercial vaccine to elicit a humoral response (Farrowsure® Gold, Serial No.: 316169Zoetis)

against Leptospirosis caused by *Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo, Leptospira icterohaemorrhagiae, and Leptospira pomona* (Wunder Jr et al., 2020). Blood samples were taken before (d 0) and after the vaccine injections on d 14, 42, 56, and 84 via jugular puncture into both heparin-coated and additive-free tubes (5 mL; BD vacutainer, BD, Mississauga, ON, Canada), centrifuged at 2500 × *g* for 15 min for plasma and serum sample collection, respectively, which were stored at -20 ℃ until further analyses.

On d 35 of the grower phase and d 77 of the finisher phase, the selected pigs (identified at the start of the experiment) representing the average BW of the pen were isolated and used for N-balance collection. The pigs were individually housed in metabolism crates (56" × 58.5") in a temperature-controlled room (21 ± 2 ℃) and assigned to the same dietary treatments as in the growth performance study. The same basal wheat-barley-soybean-based diets were used, except for the inclusion of celite (0.4%) at the expense of uncontaminated wheat. The daily feed allowed at 2.8 × maintenance net energy requirement (197 kcal/kg $BW^{0.60}$/d; NRC, 2012) and fed in two equal meals at 0700 h and 1500 h. After a 7-d dietary and environmental adaptation period, total daily urine output and fresh-fecal grab samples were collected over a 2-d period. On the last day of the 2-d collection period, approximately 3-4 h post-prandial, blood samples were taken from pigs for serum DON analysis. Pigs used in the N-balance study were not returned to the trial after the N-balance was completed.

3.2 Experimental procedure and analyses

3.2.1 Growth performance (experiment 1 and 2)

In both experiment 1 and 2, individual pig body weight and per pen feed intake was measured weekly for determination of average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (gain: feed; GF).

3.2.2 Nitrogen-balance (experiment 1 and 2)

During the sample collection days, urine jars containing sufficient HCl to maintain pH < 3 (Columbus et al., 2014) were placed under the urine collection trays of each metabolism crate to collect urine samples over two 24 h periods. At the end of each 24-h period, urine was weighed, and a 5% aliquot was sampled and stored at -20℃. At the end of the 2-d sample collection period, urine samples were thawed and pooled for each pig, filtered with glass wool to remove any debris,

and a 5% subsample was obtained and stored at -20 ºC until further analysis. Fresh fecal grab samples were taken daily by rectal palpation, pooled, homogenized at the end of the 2-d collection period, and a subsample stored at -20 ℃ until further analysis.

Table 2 Composition of the diets used in experiment 2 (as-fed basis)[1]

Ingredient, %	Grower Diet				Finisher Diet			
	CONT[2]	DON1[3]	DON3[4]	DON5[5]	CONT	DON1	DON3	DON5
Clean wheat	40.0	33.3	20.0	6.7	40.0	33.3	20.0	6.7
DON wheat [6]	0.0	4.9	14.8	24.7	0.0	4.9	14.8	24.7
Wheat screenings[7]	0.0	1.7	5.2	8.6	0.0	1.7	5.2	8.6
Barley	39.2	39.2	39.2	39.2	44.0	44.0	44.0	44.0
Canola oil	3.7	3.7	3.7	3.7	3.5	3.5	3.5	3.5
Soybean meal	14.0	14.0	14.0	14.0	10.0	10.0	10.0	10.0
L-Lysine- HCl	0.46	0.46	0.46	0.46	0.30	0.30	0.30	0.30
DL-Methionine	0.08	0.08	0.08	0.08	0.07	0.07	0.07	0.07
L-Threonine	0.14	0.14	0.14	0.14	0.10	0.10	0.10	0.10
Limestone	1.0	1.0	1.0	1.0	0.8	0.8	0.8	0.8
Dicalcium phosphate	0.8	0.8	0.8	0.8	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin/Mineral Premix[8]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Calculated nutrient content								
ME, kcal/kg	3291	3291	3291	3291	3282	3282	3282	3282
NE, kcal/kg	2495	2495	2495	2495	2502	2502	2502	2502
Dry Matter, %	86.4	86.4	86.4	86.5	86.5	86.5	86.5	86.6
Crude Protein, %	17.5	17.5	17.5	17.5	15.9	15.9	15.9	16.0
Lysine, % SID[9]	0.98	0.98	0.98	0.98	0.76	0.76	0.76	0.76
Calcium, %	0.64	0.64	0.64	0.64	0.50	0.50	0.50	0.50
Phosphorus, %	0.53	0.53	0.53	0.53	0.48	0.48	0.48	0.48
Analyzed nutrient content								
Growth performance diet								
Dry Matter, %	89.2	88.4	82.9	89.4	90.5	90.2	86.7	90.2
Crude Protein, %	15.4	17.4	16.4	16.4	16.7	16.3	17.2	16.6
DON[10], ppm[11]	0.28	0.73	3.40	4.36	0.20	1.02	3.28	4.14
Nitrogen balance diet								
Dry Matter, %	88.9	89.4	88.8	88.6	90.1	90.1	90.5	90.3
Crude Protein, %	17.8	18.8	18.2	17.3	16.0	15.7	16.5	16.5
DON, ppm	0.04	0.57	2.72	4.10	1.04	1.35	3.28	5.43

[1]Nutrient content of diets based on the nutrient content of feed ingredients according to NRC (2012).
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]DON wheat contains 6.9 ppm DON (Central Testing Laboratory, Winnipeg Manitoba)
[7]Wheat screenings contain 32.8 ppm DON (Central Testing Laboratory, Winnipeg Manitoba)
[8]Supplied per kg of complete diet; vitamin A, 8000 IU; vitamin D, 1500 IU; vitamin E, 30 IU; menadione, 2.5 mg; vitamin B12, 0.025 mg; thiamine, 1.00 mg; biotin, 0.10 mg; niacin, 20 mg; riboflavin, 4 mg; pantothenate; 12 mg; folic acid, 0.50 mg; pyridoxine, 2.0 mg; Fe, 100 mg; Zn, 100 mg; Mg, 40 mg; Cu, 15 mg; Se, 0.30 mg; and I, 1mg

[9]SID, standardized ileal digestibility
[10]DON, deoxynivalenol content analyzed by BIOMIN using high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) based analysis
[11]ppm, parts per million

3.2.3 Analysis of feed, fecal, and urine samples (experiment 1 and 2)

The dry matter (DM) content of the diet and fecal samples and were analyzed in duplicate (AOAC, 2007; Method 930.15). The N content in the diet, feces, and urine were analyzed using an automatic analyzer (LECO FP 528; MI; USA; AOAC, 2007; Method 990.03). The acid-insoluble ash (AIA) content of both diet and fecal samples were analyzed according to the method described previously (Van Keulen and Young, 1977). Nitrogen retention was calculated as the difference between nitrogen intake and nitrogen output (urine and fecal).

3.2.4 Serum chemistry panel analysis (experiment 1 and 2)

Serum samples were analyzed for key indicators of liver and kidney function and health using an automatic blood chemistry analyzer according to established methods (Prairie Diagnostic Services, Saskatoon, SK, Canada).

3.2.5 Deoxynivalenol analysis in diet, serum, and urine samples (experiment 1 and 2)

All mycotoxin analysis was performed in the laboratory of Biomin Holding GmbH (Erber Campus 1, 3131 Getzersdorf, Austria). An analytical standard for deoxynivalenol (DON) was acquired commercially (Romer Labs GmbH, Tulln, Austria). β-glucuronidase (*Escherichia coli*, Type IX-A) and PBS buffer were obtained from Sigma-Aldrich (Vienna, Austria), methanol (MeOH), and acetic acid from VWR International (Vienna, Austria), and acetonitrile from Chem-Lab NV (Zedelgem, Belgium). For the direct quantification of DON in diet, urine, and serum samples, high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) based analysis was performed according to the method described by Schwartz-Zimmermann et al., 2017. DON recovery per milliliter of urine and serum was based on actual intake.

For serum samples, indirect quantification of DON was performed. To this end, samples were analyzed both with and without β-glucuronidase pre-treatment. One hundred microliters of serum samples without β-glucuronidase was added to 200 µL of MeOH/acetic acid (99.8/0.2, v/v), shaken for 1 hour on a vortex shaker, and centrifuged (19.000 rcf, 20 min). Afterward, 200 µL of the supernatant was transferred into an HPLC vial and subjected to HPLC-MS/MS analysis. For enzymatic hydrolysis, 35 mg of β-glucuronidase was dissolved in 2.5 mL PBS and added at 50 µL per 100 µL serum. After incubation for 18 h (37° C, 80 rpm), 300 µL of MeOH/acetic acid

(99.8/0.2, v/v) were added. Subsequent shaking and centrifugation steps were performed as described above. The determination of analytes was performed on a QTRAP 6500 using an HPLC-MS/MS-based method described previously (Schwartz-Zimmermann et al., 2017). Samples were measured in duplicate and quantified using matrix-matched samples. To this end, urine samples from the control group were spiked with the standard compound at six spiking levels (0.5 – 100 ppb for DON).

3.2.6 Serum IgG titer analysis (experiment 2)

Blood serum was analyzed for *Leptospira* antibody ELISA according to Wilson-Welder et al. (2020) at the Vaccine and Infectious Disease Organization - International Vaccine Centre (Saskatoon, SK, Canada). Briefly, the detection of the immunoglobulin G (IgG) against *Leptospira spp* in pig serum was completed in a reconstituted *Leptospira* vaccine (Vanguard® L4, Zoetis Canada Inc., Kirkland, QC). Following that, a protein assay was completed to determine the protein concentration. The vaccine antigen was diluted to 10 µg/mL in a carbonate-bicarbonate buffer (pH 9.6), applied to Immulon plates (Thermo Fisher Scientific, Mississauga, ON, Canada) at 100 µL per well, covered, and incubated overnight at 4 °C. The plates were subsequently washed with 300 µL per well four times with Tris-buffered saline containing 0.05% Tween 20 (TBST). The serum samples were diluted (13 µL + 120 µL TBST) and four-fold serial dilutions across the plate for 6 wells, including a negative and positive control for each plate, and incubated for 2 h at room temperature. Plates were washed again with TBST and 100 µL of KPL Goat anti-Swine IgG (H+L) phosphatase labeled affinity purified antibody (catalog KP-15-14-06, Invitrogen, Life Technologies Inc., Burlington, ON, Canada) added at a dilution of 1/5000 in TBST and allowed to incubate at room temperature for 1h. Plates were further washed and p-nitrophenyl phosphate di(tris) salt crystalline (PNPP) (Sigma N3254, St. Louis, Missouri, United States) substrate was added at 100 µL per well and incubated for 2.5 h. Finally, the reaction was stopped by adding 30 µL 2N H_2SO_4 per well and the plate read at 405 nm, reference 490 nm on a SpectraMaxplus microplate ELISA reader (Molecular Devices, Sunnyvale, CA, USA) (da Cunha et al., 2019). The recorded absorbance values were graphed on a standard curve to calculate the IgG concentrations (mg/dL).

3.2.7 Carcass quality analysis (experiment 2)

On d 77, all pigs were transported to a commercial abattoir (Maple Leaf Foods, Brandon, MB, Canada) for slaughter and collection of carcass characteristic data, including slaughter weight (SW), backfat thickness (BFT), loin depth (LD), and overall lean yield for further analysis to assess the impact of DON on carcass yield and quality. Pigs were tattooed on a per pen basis before transporting to the packing plant. Only data for pigs correctly identified by tattoo numbers at the packing plant were utilized in the carcass analysis for increased confidence in the data even though the significant amount of data lost may also affect the results.

3.3 Statistical analyses

All data were verified for normality using the PROC UNIVARIATE (SAS Institute, Cary, NC, Version 9.4) and outliers were tested using the studentized residual analysis (values 3 standard deviation from the mean were considered outliers). The growth performance and nitrogen balance data were analyzed as a randomized complete block design with the fixed effect of dietary treatments (CONT, DON1, DON3, and DON5) and block (room) as the random variable (PROC MIXED, SAS Institute, Cary, NC, Version 9.4). The immune response data and blood chemistry analyses were analyzed as a repeated measure with 'day' as a repeated variable. Regression analysis was used to describe relationships between DON intake and ADG, total BW gain, urinary, and blood DON (PROC REG, SAS Institute, Cary, NC, Version 9.4). Significance was determined at $P < 0.05$. A trend towards significance was considered at $P \leq 0.10$. When significance is determined, the means were separated using the Tukey-Kramer mean separation test.

4.0 RESULTS

4.1 Dietary DON level in experimental diets (Expt. 1 and Expt. 2)

Before the start of the study, the mycotoxin profile was analyzed for DON-contaminated ingredients utilized (wheat and wheat screenings) (data not shown) to allow for the appropriate formulation of DON levels in the experimental diets. Subsequently, the preliminary diets formulated were also analyzed for the mycotoxin profile and concentration and also showed similar levels of DON as the calculated formulation (data not shown).

Diet samples collected during the study analyzed after completion of the study showed that the levels of DON were mostly within the formulated range except for the diets used for the N-balance study for both Expt. 1 (Table 3) and Expt. 2 (Table 4 and 5).

Table 3 Analyzed mycotoxin contents (ppm) of diets used in experiment 1 (as-fed basis)[1]

Mycotoxin, ppm	Finisher Diet							
	Growth Performance Diets				Nitrogen-Balance Diets			
	CONT[2]	DON1[3]	DON3[4]	DON5[5]	CONT	DON1	DON3	DON5
DON[6]	0.11	1.34	3.59	5.72	1.56	1.32	3.09	4.94
3 ADON[7]	ND[9]	ND	ND	ND	ND	ND	ND	ND
15 ADON[8]	ND	ND	ND	ND	ND	ND	ND	ND
HT-2 toxin	ND	ND	ND	0.05	ND	ND	ND	0.03
Nivalenol	0.15	0.18	0.53	0.64	0.12	0.11	0.12	0.08
Ochratoxin A	0.01	0.03	0.01	0.01	0.01	0.03	0.07	0.09
Zearalenone	ND	0.002	0.009	0.014	0.003	0.002	0.009	0.013
Ergot alkaloids	0.99	0.57	1.03	1.26	0.24	0.16	0.39	0.67

[1]Mycotoxin contents analyzed in diet samples by BIOMIN. All other mycotoxins were below harmful levels. These diets were made independently but from the same batch of ingredients
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]DON, deoxynivalenol
[7]3 ADON, 3-acetyldeoxynivalenol
[8]15 ADON, 15-acetyldeoxynivalenol
[9]ND, Not detected or below the limit of detection.

Table 4 Analyzed mycotoxin levels (ppm) in grower diets used in experiment 2 (as-fed basis)[1]

Mycotoxin, ppm	Grower Phase Diets							
	Growth Performance Diets				Nitrogen-Balance Diets			
	CONT[2]	DON1[3]	DON3[4]	DON5[5]	CONT	DON1	DON3	DON5
DON[6]	0.28	0.73	3.40	4.36	0.04	0.57	2.72	4.10
3 ADON[7]	ND[9]	ND	ND	ND	ND	ND	ND	ND
15 ADON[8]	ND	ND	ND	ND	ND	ND	ND	ND
HT-2 toxin	ND	ND	ND	ND	ND	ND	ND	ND
Nivalenol	0.39	0.30	0.17	0.33	0.06	0.20	0.08	0.09
Ochratoxin A	0.01	0.01	0.03	0.02	ND	0.01	0.06	0.10
Zearalenone	ND	ND	0.01	<0.01	ND	ND	<0.01	0.01
Ergot alkaloids	0.53	0.73	1.13	0.69	1.01	0.63	0.98	1.78

[1]Mycotoxin content analyzed in diet samples by BIOMIN using high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) (HPLC-MS/MS) based analysis
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]DON, deoxynivalenol
[7]3 ADON, 3-acetyldeoxynivalenol
[8]15 ADON, 15-acetyldeoxynivalenol
[9]ND, Not detected or below the limit of detection.

Table 5 Analyzed mycotoxin levels (ppm) in finisher diets used in experiment 2 (as-fed basis)[1]

Mycotoxin, ppm	Finisher Phase Diets							
	Growth Performance diets				Nitrogen-Balance diets			
	CONT[2]	DON1[2]	DON3[4]	DON5[5]	CONT	DON1	DON3	DON5
DON[6]	0.20	1.02	3.28	4.13	1.04	1.35	3.22	5.43
3 ADON[7]	ND[9]	ND	0.03	0.04	ND	ND	ND	0.02
15 ADON[8]	ND	ND	ND	ND	ND	ND	ND	ND
HT-2 toxin	ND	ND	ND	ND	ND	ND	ND	0.04
Nivalenol	0.53	0.63	0.55	0.16	0.10	0.09	0.12	0.09
Ochratoxin A	ND	0.02	0.02	0.07	0.01	0.03	0.09	0.12
Zearalenone	0.001	0.004	0.004	0.007	0.006	0.004	0.008	0.014
Ergot alkaloids	0.32	0.63	0.61	0.36	0.18	0.20	0.28	0.77

[1]Mycotoxin content analyzed in diet samples by BIOMIN using high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) (HPLC-MS/MS) based analysis
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]DON, deoxynivalenol
[7]3 ADON, 3-acetyldeoxynivalenol
[8]15 ADON, 15-acetyldeoxynivalenol
[9] ND, Not detected or below the limit of detection.

4.2 Experiment 1 (finisher pigs)

4.2.1 Growth performance

Growth performance data are presented in Table 6. Bodyweight was reduced in DON3 and DON5 fed pigs by d 7, with the greatest reduction observed in DON5 fed pigs ($P > 0.05$). This reduction in BW was consistently observed throughout the experiment. The ADG in DON1 fed pigs was not different from pigs receiving the CONT diet ($P > 0.05$). From d 0-7, DON3 fed pigs had reduced growth compared to both CONT and DON1 fed pigs ($P < 0.05$), which was not different from CONT fed pigs from d 8-42 ($P > 0.05$). Pigs fed DON5 had reduced ADG from d 0-21 compared to all other dietary treatments ($P < 0.05$). From d 22-28, ADG of DON5 fed pigs was not different than DON1 and DON3 fed pigs *(*$P > 0.05$). From d 29-42, there were no differences in ADG among dietary treatments ($P > 0.05$). Overall (d 0-42), ADG was reduced in DON3 and DON5 fed pigs compared to both CONT and DON1, with the greatest reduction observed with DON5 ($P < 0.05$). There was no impact of DON1 on ADFI compared to CONT ($P > 0.05$). From d 0-7, DON3 fed pigs had reduced ADFI compared to both CONT and DON1 fed pigs ($P < 0.05$), after which no difference was observed ($P > 0.05$). In DON5 fed pigs, ADFI was reduced from d 0-28 compared to all other dietary treatments ($P < 0.05$), after which no difference was observed ($P > 0.05$). Overall (d 0-42), ADFI was only reduced in DON5 fed pigs ($P < 0.05$). Feed efficiency, measured as GF, was reduced in DON5 fed pigs from d 0-7 compared to all other dietary treatments ($P < 0.05$), which were not different from each other ($P > 0.05$). There was no effect of dietary treatment on GF from 8-42 or overall (d 0-42; $P > 0.05$).

4.2.2 Relationship between dietary DON intake and body weight gain

A linear regression model was applied to examine the relationship between DON intake and growth (% BW gain, Fig. 1; ADG, Fig. 2). For this analysis, the BW gain of all DON-contaminated treatments (DON1, DON3, and DON5) was compared to the average BW gain of the CONT pigs. As indicated, there was a negative relationship between DON intake and BW gain, when DON intake increased, % BW gain was reduced. This negative relationship was also observed for ADG, such that as DON intake increased, a linear reduction in ADG was observed. This relationship was consistent from d 0-35, however, the strength of the impact of DON intake on ADG was reduced ($P < 0.05$) consistently and from d 28-35, the impact was lowest, with a

slope not different from zero (P > 0.05; Fig.2E). From d 35-42, the slope shows a positive relationship (Fig. 2F).

Table 6 Growth performance of finisher pigs fed graded levels of deoxynivalenol[1]

	Dietary Treatments					
Items	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P-value*
Body Weight, kg						
Initial	76.9	77.0	76.3	76.0	1.18	NS[7]
Day 7	85.4[a]	84.8[a]	83.0[b]	80.8[c]	0.34	<0.001
Day 14	95.3[a]	95.3[a]	92.4[b]	88.7[c]	0.42	<0.001
Day 21	103.4[a]	103.8[a]	99.8[b]	95.7[c]	0.50	<0.001
Day 28	112.1[a]	111.9[a]	107.8[b]	103.0[c]	0.53	<0.001
Day 35	119.7[a]	119.8[a]	114.9[b]	110.4[c]	0.63	<0.001
Final	126.7[a]	126.9[a]	123.6[b]	118.5[c]	0.80	<0.001
Average daily gain, kg/d						
Day 0-7	1.27[a]	1.18[a]	0.93[b]	0.60[c]	0.05	<0.001
Day 8-14	1.40[ab]	1.49[a]	1.33[b]	1.13[c]	0.04	<0.001
Day 15-21	1.17[ab]	1.21[a]	1.06[b]	1.01[c]	0.04	0.004
Day 22-28	1.24[a]	1.17[ab]	1.15[ab]	1.04[b]	0.04	0.033
Day 29-35	1.08	1.12	1.01	1.06	0.04	NS
Day 36-42	1.06	1.00	1.20	1.14	0.06	NS
Overall (d 0-42)	1.19[a]	1.20[a]	1.12[b]	1.00[c]	0.02	<0.001
Average daily feed intake, kg/d						
Day 0-7	2.59[a]	2.59[a]	2.22[b]	1.70[c]	0.06	<0.001
Day 8-14	2.98[a]	3.07[a]	2.89[a]	2.55[b]	0.07	<0.001
Day 15-21	3.03[a]	3.03[a]	2.88[a]	2.56[b]	0.05	<0.001
Day 22-28	3.25[a]	3.19[a]	3.13[a]	2.85[b]	0.05	<0.001
Day 29-35	3.22	3.20	3.19	3.04	0.06	NS
Day 36-42	3.19	3.11	3.36	3.05	0.08	NS
Overall (d 0-42)	2.99[a]	3.06[a]	2.94[a]	2.60[b]	0.05	<0.001
Gain: Feed, kg/kg						
Day 0-7	0.49[a]	0.46[a]	0.41[a]	0.34[b]	0.02	<0.001
Day 8-14	0.47	0.49	0.47	0.44	0.01	NS
Day 15-21	0.38	0.40	0.37	0.40	0.01	NS
Day 22-28	0.38	0.36	0.37	0.36	0.02	NS
Day 29-35	0.33	0.35	0.32	0.35	0.01	NS
Day 36-42	0.33	0.32	0.36	0.37	0.01	NS
Overall (d 0-42)	0.40	0.39	0.38	0.38	0.01	NS

[1]Values are least-squares means (n=10 pens/treatment). Data were analyzed as a randomized complete block design. Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$.
[2]CONT, 0 ppm DON Control diet

[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]SEM, Standard error of means
[7]NS, Not significant ($P > 0.05$)
[a, b, c] Means without a common superscript within a row are significantly different.

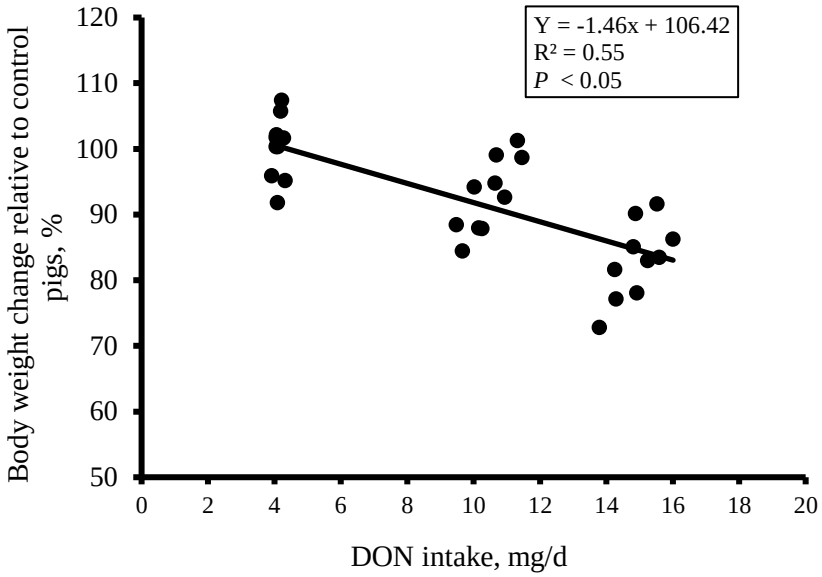

Figure 1 Regression analysis showing the relationship between deoxynivalenol (DON) intake and body weight change (%) relative to control (no DON) fed pigs. Each data point represents a pen (n=10 pens/treatment). Data expressed as the DON intake (mg/d) based on average daily feed intake (ADFI) and the bodyweight change of the DON treated groups relative to the control (CONT) group of pigs. The coefficient of determination (R^2) of the regression curve is 0.55 at $P < 0.05$.

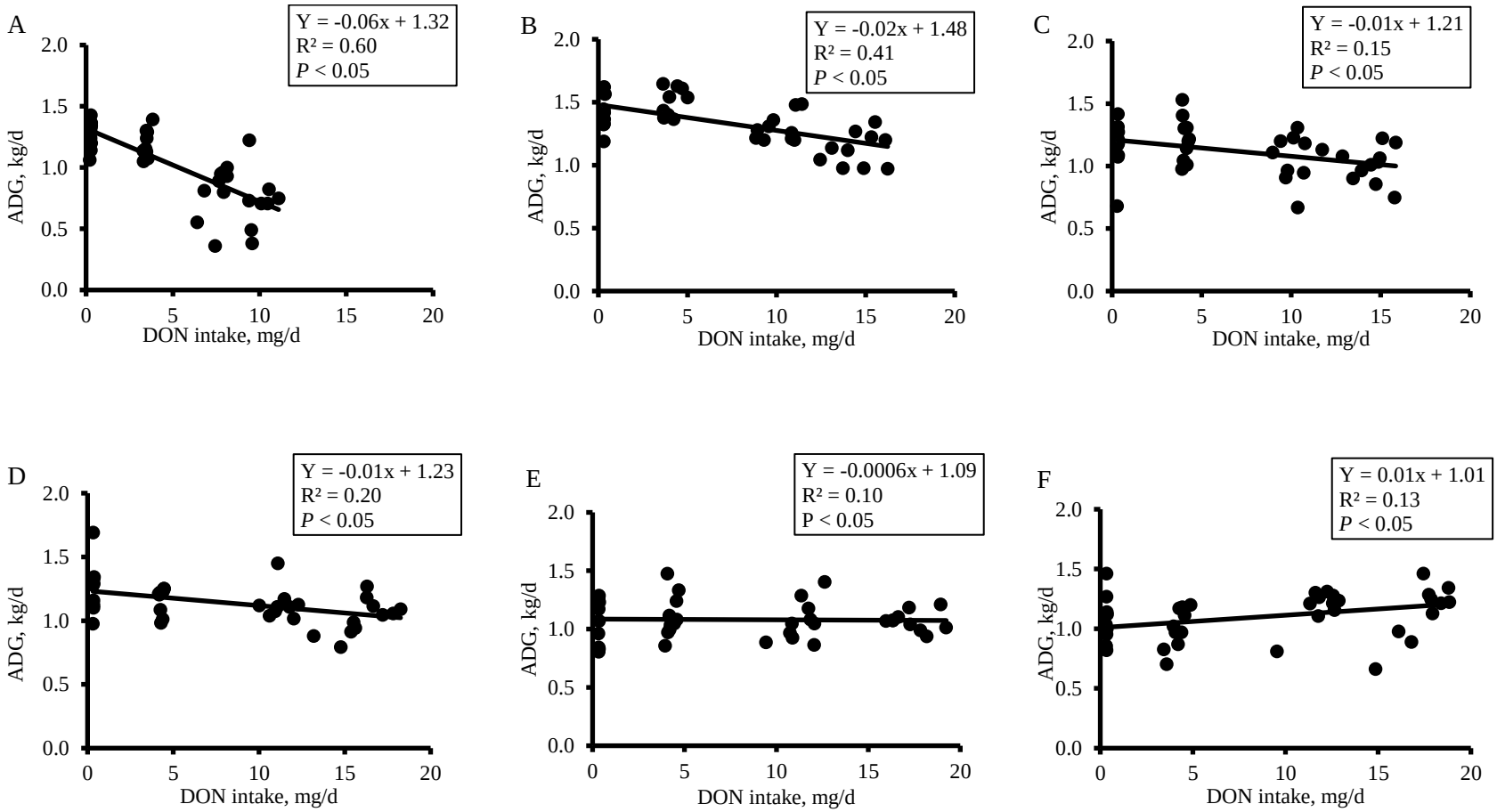

Figure 2 Regression analysis showing the relationship between deoxynivalenol (DON) intake and average daily gain (ADG) in finisher pigs from d 0-7 (A), d 8-14 (B), d 15-21 (C), d 22-28 (D), d 29-35 (E), and d 36-42 (F). Each point on the graph represents an experimental pen (n=10 pens/treatment). Data are expressed as the DON intake (mg/d) based on average daily feed intake and the average daily gain (ADG). The coefficient of determination (R^2) of the regression curve is 0.60, 0.41, 0.15, 0.20, 0.10, and 0.13 respectively for A, B, C, D, E, and F, respectively, at $P < 0.05$.

4.2.3 Nitrogen-balance

The results for the N-balance are presented in Table 7. Average daily N intake for pigs fed DON3 and DON5 were not different but both were lower ($P < 0.05$) than DON1 and CONT diet. The urinary N output from CONT, DON3, and DON5 diets were not different ($P > 0.05$) but the pigs fed DON1 had lower ($P < 0.05$) urinary N output compared to DON3 and CONT but not different from DON5. The fecal N output was not different between DON1, DON3, and DON5, but was higher ($P < 0.05$) in the CONT diet. The apparent total tract digestibility (ATTD) of N was lower ($P < 0.05$) in the CONT diet compared to all the other dietary treatments. The protein deposition (PD) for DON1 pigs, which received the lowest DON content in their diets, was higher ($P < 0.05$) than pigs fed CONT, DON3, and DON5 diets.

4.2.4 Liver and kidney metabolites

Key indicators of kidney and liver health and function are presented in Table 8. There was no effect ($P > 0.05$) of dietary DON content on the selected liver and kidney blood parameters. For most of the analyzed metabolites there was a significant effect of day, except for potassium and creatine kinase, which tended to be different ($P = 0.075$ and 0.073, respectively), and gamma-glutamyl transferase, which was not affected ($P > 0.05$) by day. These differences are considered to not be related to dietary treatment as there was no significant diet × day interaction.

4.2.5 Concentration of DON in serum and urine

A linear regression model was used to analyze the relationship between DON intake and DON and DON metabolite concentration in blood serum and recovery in urine as shown in Fig. 3, 4, and 5. As dietary DON intake increased, the amount of DON in the blood increased. For urinary DON analysis, as dietary DON levels increased, there was an increase ($P < 0.05$) in DON in the urine. DON1 pigs also showed lower ($P = 0.02$) DON recovery in urine compared to DON3 but not different from DON5.

Table 7 Nitrogen-balance in pigs fed diets containing graded levels of deoxynivalenol for 42 d [1]

	Dietary Treatments					
Items	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P-value*
N[7] intake, g/d	67.84[a]	68.45[a]	63.48[b]	63.32[b]	1.27	0.024
ATTD[8] of N, %	79.27[a]	87.12[b]	85.41[b]	85.44[b]	1.56	0.009
Urinary N output, g/d	25.53[a]	15.19[b]	28.38[a]	22.69[ab]	3.59	0.015
Fecal N output, g/d	14.10[a]	8.82[b]	9.28[b]	9.26[b]	1.05	0.005
N retained, g/d	28.30[b]	44.40[a]	25.85[b]	31.34[b]	3.17	<0.001
N retained, %	41.68[b]	65.12[a]	40.62[b]	49.64[b]	4.75	<0.001
Protein deposition[9], g/d	176.89[b]	277.78[a]	161.56[b]	195.99[b]	19.81	<0.001

[1]Values are least-squares means (n=10 pens/treatment). Data were analyzed as a randomized complete block design. Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$.
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of means.
[7]N, nitrogen
[8]ATTD, Apparent total tract digestibility
[9]Protein deposition = N retained × 6.25
[a, b] Means without a common superscript within a row are significantly different ($P < 0.05$)

Table 8 Serum chemistry panel for liver and kidney metabolites[1]

Item	Day	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P*-value		
							Diet	Day	Diet × Day
Sodium, mM	0	144.4	144.1	144.5	144.5	0.70			
	14	146.2	145.9	146.1	145.8	0.70	NS[7]	<.001	NS
	42	145.0	146.3	145.9	145.2	0.70			
Potassium, mM	0	5.45	5.42	5.35	5.60	0.18			
	14	5.32	5.05	5.01	5.44	0.18	NS	NS	NS
	42	5.21	5.32	5.28	5.32	0.18			
Chloride, mM	0	97.4	97.9	97.3	97.4	0.70			
	14	98.9	99.7	99.1	99.6	0.70	NS	<.0001	NS
	42	98.8	99.9	99.6	98.9	0.70			
Bicarbonate, mM	0	26.8	26.9	28.4	27.3	0.71			
	14	31.1	29.3	29.3	28.8	0.71	NS	<.0001	NS
	42	33.4	32.3	33.1	32.2	0.71			
Anion Gap, mM	0	25.9	24.4	24.2	25.4	1.03			
	14	21.6	21.9	22.7	22.9	1.03	NS	<.0001	NS
	42	18.1	19.4	18.4	19.5	1.03			
Calcium, mM	0	2.84	2.78	2.82	2.86	0.05			
	14	2.82	2.74	2.74	2.80	0.05	NS	0.01	NS
	42	2.75	2.95	2.92	2.88	0.05			
Phosphorus, mM	0	2.99	2.97	3.03	3.00	0.90			
	14	3.05	3.06	3.05	3.14	0.90	NS	<.0001	NS
	42	2.52	2.52	2.54	2.58	0.90			
Magnesium, mM	0	0.94	0.90	0.93	0.94	0.02			
	14	0.90	0.90	0.89	0.90	0.02	NS	<.0001	NS
	42	0.84	0.84	0.84	0.85	0.02			

Table 8 (continued) Serum chemistry panel for liver and kidney metabolites[1]

Urea, mM	0	4.03	3.96	3.64	3.70	0.30			
	14	4.74	4.40	4.09	4.12	0.30	NS	<.0001	NS
	42	4.91	4.81	5.00	4.67	0.30			
Creatinine, mM	0	112.4	113.6	111.9	114.5	4.60			
	14	118.5	118.9	116.8	122.3	4.60	NS	<.0001	NS
	42	136.6	139.4	134.9	140.6	4.60			
Glucose, mM	0	5.53	5.68	5.83	5.86	0.18			
	14	5.54	5.30	5.45	5.44	0.18	NS	0.05	NS
	42	5.34	5.69	5.65	5.21	0.18			
Total Bilirubin, mM	0	1.10	1.10	1.09	1.17	0.10			
	14	0.72	0.84	0.86	0.73	0.10	NS	<.0001	NS
	42	0.39	0.50	0.43	0.44	0.10			
Direct Bilirubin, mM	0	0.44	0.38	0.42	0.37	0.04			
	14	0.27	0.27	0.25	0.25	0.04	NS	<.0001	NS
	42	0.25	0.33	0.25	0.28	0.04			
Indirect Bilirubin, mM	0	0.65	0.73	0.67	0.79	0.10			
	14	0.44	0.58	0.61	0.47	0.10	NS	<.0001	NS
	42	0.13	0.18	0.18	0.15	0.10			
GGT[8], mM	0	39.7	36.8	41.6	38.9	3.40			
	14	36.9	35.5	37.2	42.3	3.40	NS	NS	NS
	42	34.5	34.5	37.9	38.9	3.40			
GLDH[9], mM	0	1.7	1.6	1.4	1.3	0.38			
	14	1.9	1.7	2.0	2.8	0.38	NS	<.01	NS
	42	1.2	1.2	1.2	1.2	0.38			
AST[10], mM	0	30.3	28.2	23.0	25.9	3.10			
	14	25.6	29.3	25.5	33.5	3.10	NS	<.0001	NS
	42	19.9	18.9	17.4	19.5	3.10			

Table 8 (continued) Serum chemistry panel for liver and kidney metabolites[1]

CK[11], mM	0	3134	1559	144	2035	698.4			
	14	2545	3315	2208	3615	698.4	NS	NS	NS
	42	2579	1885	1116	2390	698.4			
Total Protein, mM	0	68.0	63.9	63.3	64.1	1.70			
	14	65.4	63.7	60.7	63.6	1.70	NS	<.01	NS
	42	63.4	61.9	61.7	63.3	1.70			
Albumin, mM	0	43.1	42.0	42.4	43.7	0.90			
	14	41.4	41.5	41.0	42.3	0.90	NS	0.005	NS
	42	42.0	43.0	43.3	43.5	0.90			
Globulin, mM	0	24.9	21.9	20.9	20.4	1.90			
	14	24.0	22.2	19.7	21.3	1.90	NS	0.001	NS
	42	21.4	18.9	18.3	19.8	1.90			
Albumin:Globulin, mM	0	1.93	2.04	2.14	2.18	0.18			
	14	1.9	2.0	2.2	2.0	0.18	NS	0.002	NS
	42	2.1	2.4	2.5	2.3	0.18			

[1]Values are least-squares means (n=10/treatment); DON, deoxynivalenol. The data was analyzed as a repeated measure with 'day' as the repeated variable. Significance determined at $P < 0.05$ and a trend towards significant at $0.05 < P < 0.10$.
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of means.
[7]NS, Not significant ($P > 0.05$)
[8]GGT, Gamma-glutamyltransferase
[9]GLDH, Glutamate dehydrogenase
[10]AST, Aspartate aminotransferase
[11]CK, Creatine kinase

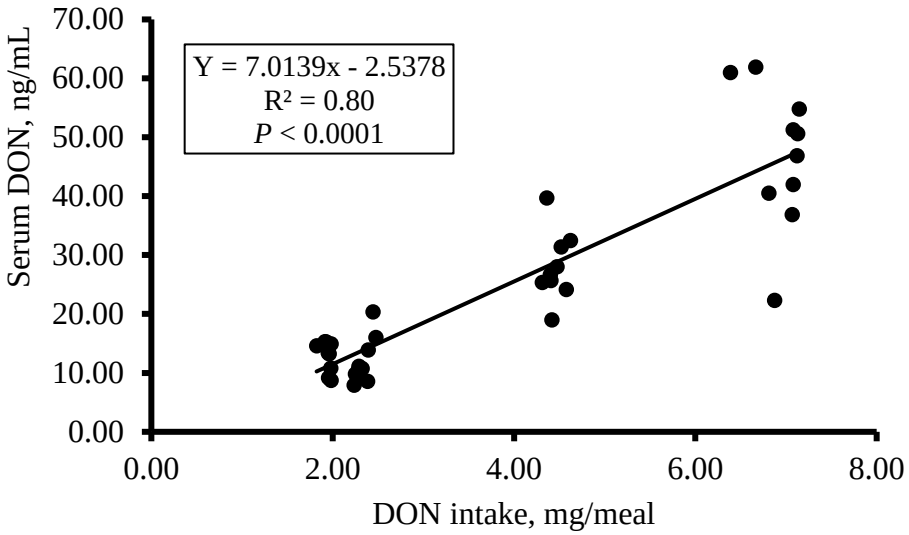

Figure 3 Regression analysis of the relationship between deoxynivalenol (DON) intake and serum DON concentration (ng/mL). The blood samples were taken during the nitrogen balance period (3-4 hours after a single meal) of the experiment and analyzed for DON concentration (n=10 pigs/treatment). Data are expressed as the DON intake after a single meal and the serum DON concentration (ng/mL) after that meal. The coefficient of determination (R^2) of the regression curve is 0.80 at $P < 0.0001$.

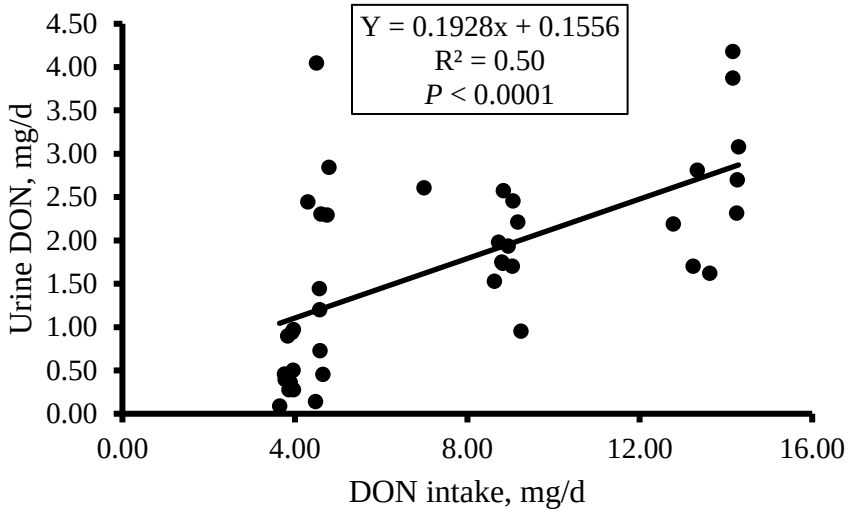

Figure 4 Regression analysis of the relationship between deoxynivalenol (DON) intake and DON output in urine (n=10 pigs/treatment).The urine samples were collected during the nitrogen balance period of the experiment over 24-h and analyzed for DON content. Data are expressed as the DON intake per day (mg/d) and the urine DON output (mg/d) and the coefficient of determination (R^2) of the regression curve is 0.50 at $P < 0.0001$.

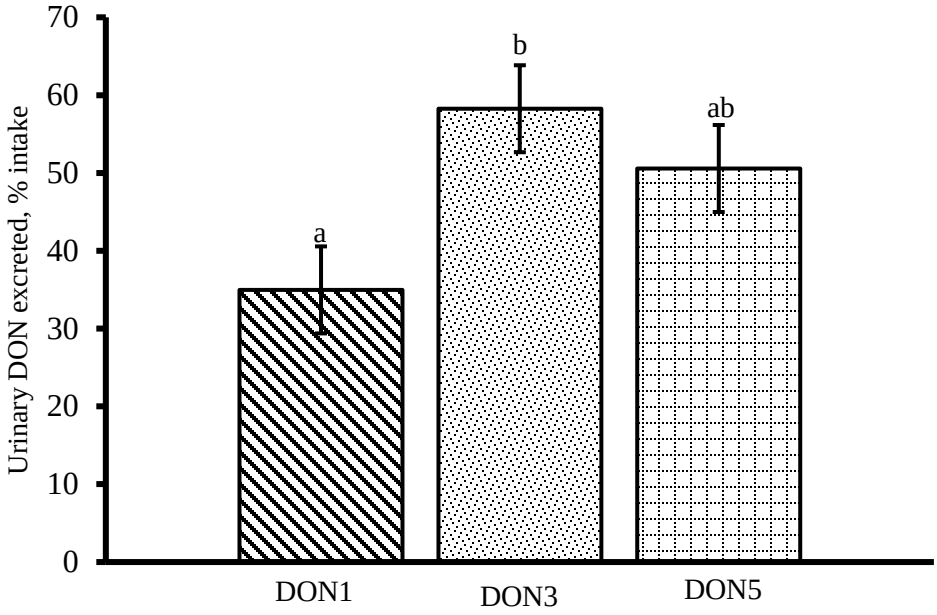

Figure 5 Deoxynivalenol (DON) recovery in urine expressed as a percentage of DON intake per pig per d in finisher pigs fed diets containing 1, 3, or 5 ppm DON (DON1, DON3, DON5, respectively). Bars represent urinary DON recovery shown as least square means ± SEM (n=10 pigs/treatment). Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$.

4.3 Experiment 2 (grower-finisher pigs)

4.3.1 Growth performance and carcass characteristics

The growth performance results are presented in Table 9. The initial BW was not different ($P > 0.05$) between the dietary treatments. Pigs fed DON5 had reduced BW by d 21 and DON3 fed pigs by d 35 compared to CONT fed pigs ($P < 0.05$) and in general this observation remained for the duration of the study. There was no difference in BW between CONT and DON1 at any point ($P > 0.05$). From d 0-7, the ADG for pigs fed the DON3 and DON5 diets were lower ($P < 0.05$) than DON1 and CONT fed pigs. No effect ($P > 0.05$) of increasing dietary DON levels on ADG was observed from d 7-42. From d 42-49, ADG for pigs fed the DON5 diet was reduced ($P > 0.05$) compared to the other dietary treatments. Subsequently, no further treatment effects were observed ($P > 0.05$) on ADG from d 49-77. Only pigs fed the DON5 and DON3 diets had reduced ADG compared to CONT but no difference in ADG between DON1 entire grower phase (d 0-42) and no treatment differences observed over the entire finisher phase (d 42-77). Over the entire growth performance period (d 0-77), both DON3 and DON5 resulted in reduced ADG ($P < 0.05$) compared to both CONT and DON1, which were not different ($P > 0.05$).

From d 0-7, the ADFI of pigs fed the DON5 diet was lower ($P < 0.05$) compared to pigs fed CONT, DON1, and DON3 diets. From d 7-21, there was no impact on dietary treatment ($P > 0.05$) on ADFI. However, from d 21-28, pigs fed DON-contaminated diets (DON1, DON3, and DON5) had a significantly higher ADFI ($P < 0.05$) compared to the CONT fed pigs. From d 28-42, no significant differences in ADFI were observed ($P > 0.05$). Over the entire grower period (d 0-42) there was no impact of diet on ADFI ($P < 0.05$) whereas in the finisher period (d 42-77), feeding of DON-contaminated diets reduced ADFI compared to CONT ($P < 0.05$). Over the entire growth performance study, ADFI was reduced ($P < 0.05$) in DON3 and DON5 fed pigs compared to CONT and DON1, which were not different ($P > 0.05$). There was no impact of dietary treatment on GF ($P > 0.05$). Carcass characteristics data are presented in Table 10. There was no significant effect ($P > 0.05$) of dietary treatments on parameters of carcass characteristics as measured.

Table 9 Growth performance of grower-finisher pigs fed diets with graded levels of deoxynivalenol[1]

	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	P-value
Bodyweight, kg						
Initial	36.0	35.6	35.7	36.4	0.34	NS[7]
Day 7	42.5	41.6	40.7	41.7	0.44	NS
Day 14	50.1^{a}	49.8^{a}	47.8^{b}	49.2^{ab}	0.49	0.010
Day 21	58.0^{a}	57.7^{a}	55.7^{ab}	56.7^{b}	0.60	0.040
Day 28	68.1	67.6	65.4	65.7	0.84	NS
Day 35	75.9^{a}	74.5^{ab}	72.7^{b}	72.7^{b}	0.86	0.030
Day 42	85.2^{a}	83.7^{ab}	81.9^{b}	81.6^{b}	0.91	0.030
Day 49	94.7^{a}	93.1^{ab}	90.9^{bc}	89.8^{c}	0.96	0.005
Day 56	102.7^{a}	100.9^{ab}	98.3^{bc}	97.7^{c}	1.00	0.004
Day 63	110.6^{a}	108.6^{ab}	106.3^{bc}	105.0^{c}	0.91	0.001
Day 70	118.4^{a}	116.2^{ab}	114.6^{bc}	112.9^{c}	0.91	0.001
Final	124.9^{a}	123.0^{ab}	121.0^{bc}	120.0^{c}	0.91	0.002
Average daily gain, kg/d						
Day 0-7	0.92^{a}	0.86^{a}	0.72^{b}	0.76^{b}	0.04	0.001
Day 8-14	1.09	1.17	1.02	1.08	0.04	NS
Day 15-21	1.14	1.13	1.12	1.06	0.03	NS
Day 22-28	1.44	1.42	1.38	1.30	0.06	NS
Day 29-35	1.15	1.12	1.14	1.11	0.04	NS
Day 36-42	1.32	1.32	1.32	1.27	0.04	NS
Day 43-49	1.37^{a}	1.34^{a}	1.28^{a}	1.17^{b}	0.04	0.010
Day 50-56	1.13	1.11	1.05	1.13	0.06	NS
Day 57-63	1.13	1.11	1.15	1.04	0.05	NS
Day 63-70	1.13	1.08	1.18	1.13	0.04	NS
Day 71-77	0.93	1.03	0.91	1.00	0.06	NS
Day 0-42	1.17^{a}	1.15^{ab}	1.10^{bc}	1.08^{c}	0.02	0.010
Day 43-77	1.14	1.13	1.11	1.10	0.01	NS
Overall (d 0-77)	1.15^{a}	1.14^{a}	1.11^{b}	1.09^{b}	0.01	0.001

Table 9 (continued) Growth performance of grower-finisher pigs fed diets with graded levels of deoxynivalenol[1]

Average daily feed intake, kg/d						
Day 0-7	1.59[a]	1.55[a]	1.40[b]	1.42[b]	0.04	0.002
Day 8-14	1.90	1.98	1.78	1.81	0.07	NS
Day 15-21	2.03	1.95	1.93	1.95	0.06	NS
Day 22-28	2.37[b]	2.58[a]	2.49[a]	2.49[a]	0.03	0.002
Day 29-35	2.79	2.77	2.67	2.60	0.05	NS
Day 36-42	3.17	3.07	3.09	2.95	0.08	NS
Day 43-49	3.17[a]	2.95[a]	2.96[a]	2.71[b]	0.08	0.004
Day 50-56	3.19[a]	3.06[ab]	2.99[b]	2.94[b]	0.06	0.01
Day 57-63	3.02	2.80	2.89	2.88	0.09	NS
Day 63-70	3.19	3.05	3.06	2.97	0.05	NS
Day 71-77	3.05	2.99	2.94	2.91	0.07	NS
Day 0-42	2.29	2.27	2.20	2.18	0.03	NS
Day 43-77	3.12[a]	2.97[b]	2.96[b]	2.88[b]	0.05	0.001
Overall (d 0-77)	2.62[a]	2.55[ab]	2.47[b]	2.47[b]	0.03	0.003
Gain: Feed, kg/kg						
Day 0-7	0.58	0.56	0.52	0.53	0.02	NS
Day 7-14	0.58	0.60	0.58	0.60	0.02	NS
Day 15-21	0.57	0.58	0.59	0.55	0.02	NS
Day 22-28	0.60	0.55	0.56	0.52	0.02	NS
Day 29-35	0.41	0.41	0.43	0.43	0.01	NS
Day 36-42	0.42	0.43	0.43	0.43	0.01	NS
Day 43-49	0.43	0.46	0.43	0.44	0.02	NS
Day 50-56	0.35	0.36	0.35	0.38	0.02	NS
Day 57-63	0.37	0.41	0.40	0.36	0.02	NS
Day 63-70	0.35	0.36	0.39	0.38	0.01	NS
Day 71-77	0.31	0.35	0.31	0.35	0.02	NS
Day 0-42	0.51	0.50	0.50	0.49	0.01	NS
Day 43-77	0.35	0.36	0.39	0.38	0.01	NS
Overall (d 0-77)	0.44	0.45	0.45	0.44	0.004	NS

[1]Values are least-squares means (n=10 pens/treatment). Data were analyzed as a randomized complete block design. Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$.
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of means
[7]NS, Not significant ($P > 0.05$)
[a, b, c] Means without a common superscript within a row are significantly different.

Table 10 Carcass characteristics for pigs fed diets containing graded levels of DON for 77 d[1]

Item	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P-value*
pigs/treatment	31	37	37	39		
Live weight, kg	122.65	121.48	119.42	116.68	1.62	NS[7]
Slaughter weight, kg	99.49	97.08	96.22	94.85	1.32	NS
Backfat thickness, mm	16.54	15.68	15.93	16.20	0.62	NS
Loin depth, mm	67.95	67.39	65.15	65.33	0.99	NS
Yield, %	61.91	62.36	62.07	61.97	0.30	NS
Dressing, %	81.40	80.00	80.56	81.29	1.07	NS

[1]Values are least-squares means (n=10 pens/treatment). Data were analyzed as a randomized complete block design. Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$.
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]SEM, Standard error of means
[7]NS, Not significant ($P > 0.05$)

4.3.2 Relationship between dietary DON intake and body weight gain

The relationship between dietary DON intake and body weight gain was evaluated using a linear regression model as shown in Fig. 7. In general, there was a negative relationship between DON intake and ADG in the grower phase and over the entire growth performance study, however, there was only a weak relationship in the finisher phase. The relationship between DON intake and body weight gain of the treatment groups (DON1, DON3, and DON5) relative to CONT is presented in Fig. 6. The results indicated that an increase in DON intake reduced the rate of BW gain relative to the CONT pigs. Further, an increase in DON intake increased the magnitude of BW change relative to the CONT in a linear trend. The observation above was present throughout the grower phase (d 0-42) and by the end of the finisher phase (d 42-77).

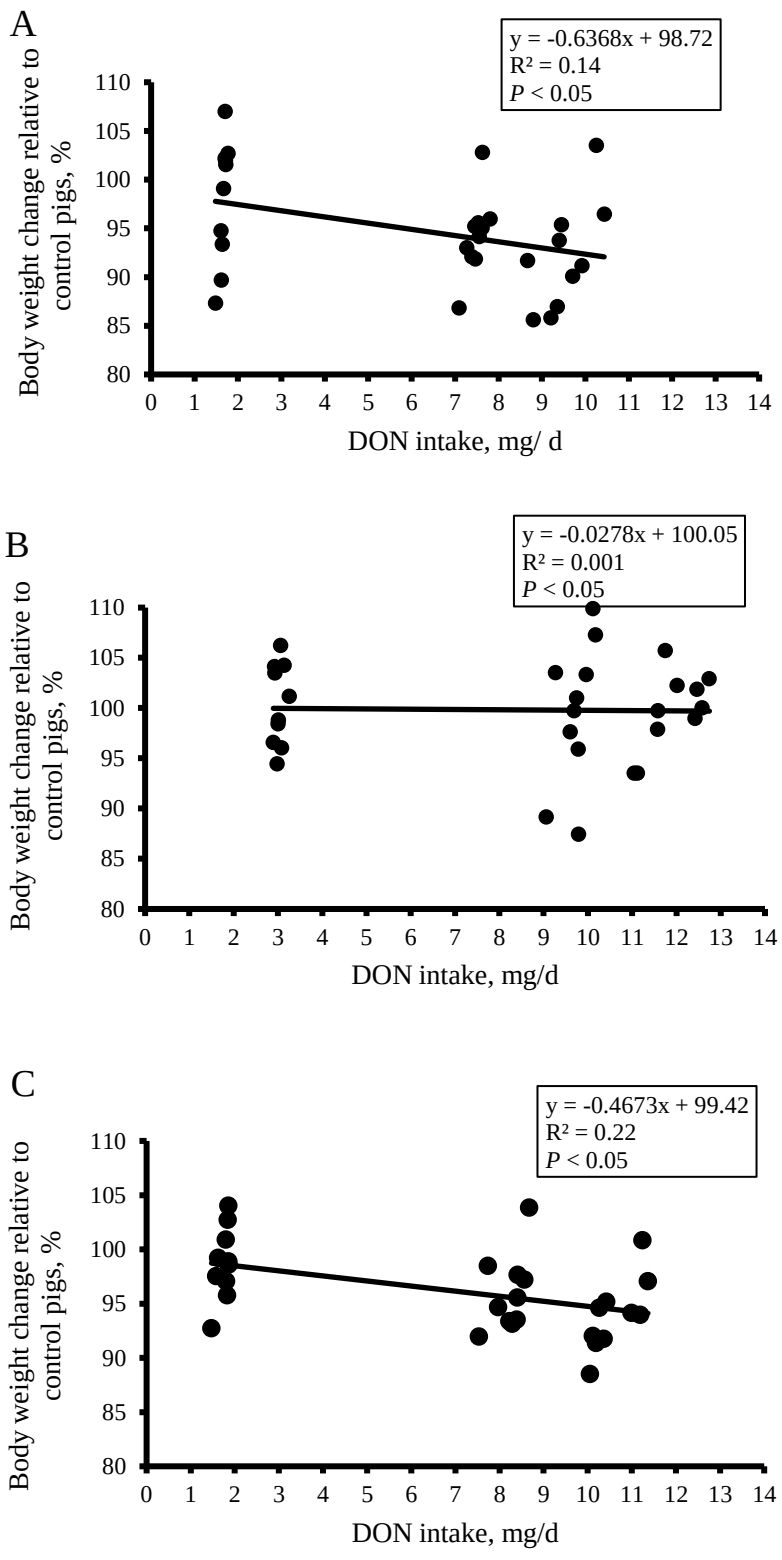

Figure 6 Regression analysis showing the relationship between deoxynivalenol (DON) intake and body weight change (%) relative to control (no DON) fed pigs during the grower phase d 0-42 (A), finisher phase d 42–77 (B), and overall d 0–77 (C). Each data point represents a pen (n=10

pens/treatment). Data expressed as the DON intake (mg/d) based on average daily feed intake and the bodyweight change of the DON treated groups relative to the control (CONT) group of pigs. The coefficient of determination (R^2) are 0.14, 0.001, and 0.22 for figure A (grower phase), figure B (finisher phase), and figure C (overall), respectively, at $P < 0.05$.

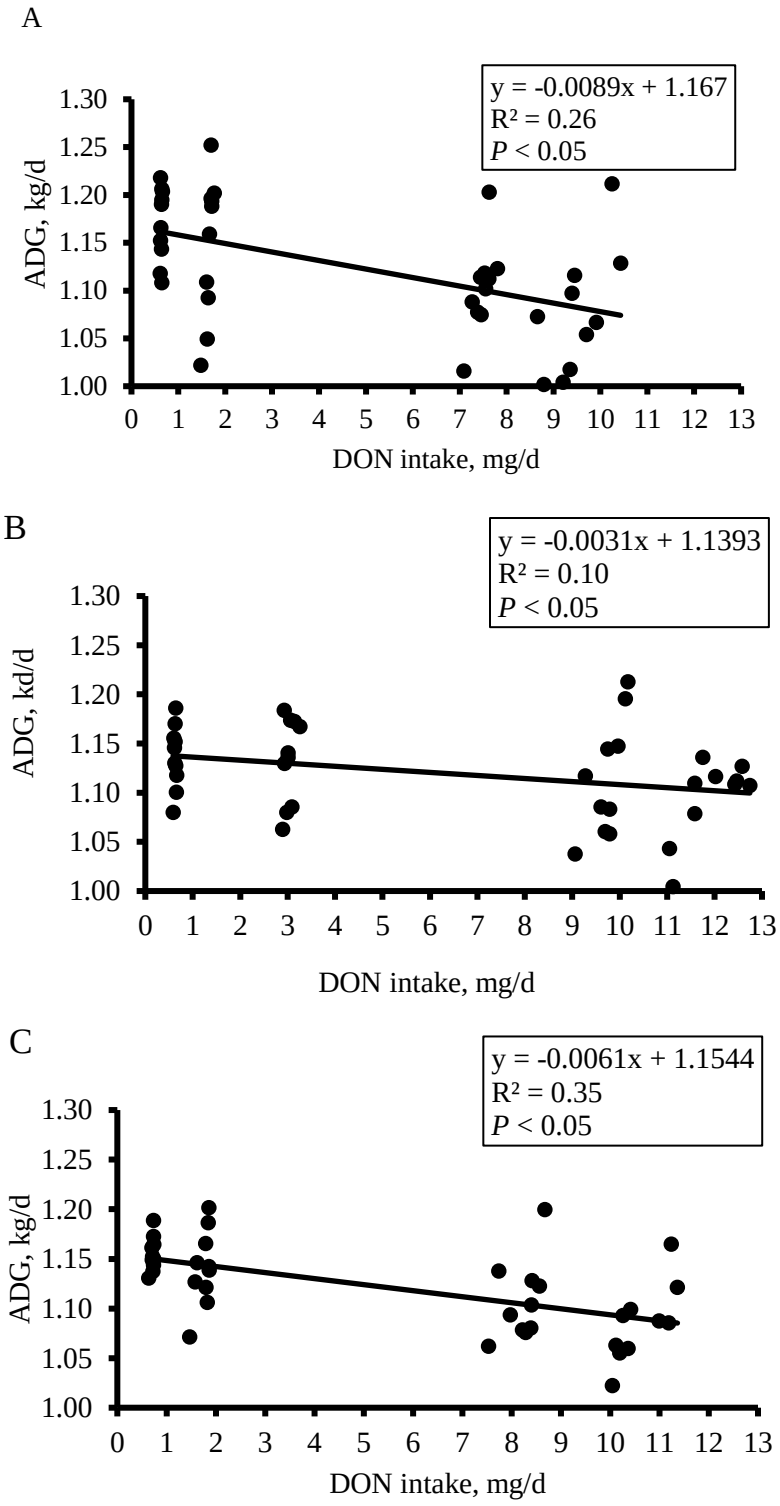

Figure 7 Regression analysis of the relationship between deoxynivalenol (DON) intake and average daily gain (ADG) in pigs fed DON-contaminated diets during the grower phase d 0-42 (A), finisher phase d 42-77 (B), and overall d 0-77 (C). Each point on the graph represents an experimental pen (n=10 pens/treatment). Data are expressed as the DON intake (mg/d) based on average daily feed intake and the bodyweight change of the DON treated groups relative to the control (CONT) group of pigs. The coefficient

of determination (R^2) of the regression curve is 0.26, 0.10, and 0.35 for figure A (grower phase), figure B (finisher phase), and figure C (overall), respectively, at $P < 0.05$.

4.3.3 Nitrogen balance

Nitrogen-balance results are presented in Table 11. In the grower phase, the N-intake of pigs fed DON3 and DON5 diets was lower ($P < 0.05$) compared to pigs fed CONT and DON1 diets. Digestibility of N was higher ($P < 0.05$) for CONT and DON3 pigs compared to DON1-fed pigs but not different from DON5 fed pigs and fecal N output was higher ($P < 0.05$) in DON1 fed pigs compared to all other treatments. Urinary N output was not affected ($P > 0.05$) by dietary treatments. Nitrogen retention and PD were reduced ($P < 0.05$) in pigs fed DON3 and DON5 compared to CONT and DON1 fed pigs, which were not different. In the finisher phase, N intake was higher ($P < 0.05$) in DON1 fed pigs compared to CONT and DON3, but not different from DON5. Nitrogen digestibility was lower ($P < 0.05$) in all DON treatments (DON1, DON3, DON5) compared to CONT fed pigs resulting in increased fecal N output for all DON treatments. Urinary N output was not affected ($P < 0.05$) by dietary treatment. Overall, nitrogen retention and PD were not affected by dietary treatment in the finisher period ($P > 0.05$).

4.3.4 Liver and kidney metabolites

Selected indicators of kidney and liver health and function are presented in Table 12 and Table 13 respectively for the grower and finisher phases of the study. Generally, no significant effects ($P > 0.05$) of dietary DON on the selected liver and kidney metabolites were observed.

4.3.5 Concentration of DON in serum and urine

A linear regression model showed a significant positive relationship ($P < 0.05$) between dietary DON intake and DON concentration in serum (in both grower and finisher phases; Fig. 8) and recovery in urine (in both grower and finisher phases; Fig. 9). There were also no significant differences ($P > 0.05$) in urinary DON excretion (Fig. 10) for both the grower and finisher phases.

Table 11 Nitrogen balance results for pigs fed deoxynivalenol contaminated diets[1]

	Dietary Treatment					
Items	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P-value*
Grower pigs (d 0 - 42)						
N[7] intake, g/d	68.2[a]	66.9[a]	62.6[b]	58.6[b]	1.57	0.001
ATTD[8], of N, %	84.9[a]	79.9[b]	83.7[a]	82.7[ab]	0.92	0.001
Urinary N output, g/d	21.6	21.6	21.5	20.6	1.50	NS[9]
Fecal N output, g/d	10.3[b]	13.4[a]	10.2[b]	10.1[b]	0.47	0.001
N retained, g/d	36.4[a]	31.9[ab]	30.9[b]	27.8[b]	1.75	0.001
N retained, %	53.3	47.7	49.2	47.7	2.17	NS
Protein deposition[10],g/d	227.2[a]	199.9[ab]	193.2[b]	173.7[b]	10.94	0.001
Finisher pigs (d 42-77)						
N[7] intake, g/d	77.9[b]	83.7[a]	75.2[b]	80.4[ab]	1.41	0.001
ATTD[8], of N, %	91.2[a]	88.1[b]	87.6[b]	87.8[b]	0.52	0.001
Urinary N output, g/d	35.7	32.5	31.5	34.5	1.86	NS
Fecal N output, g/d	6.9[b]	9.9[a]	9.4[a]	9.8[a]	0.46	0.001
N retained, g/d	35.4	41.3	34.2	36.0	2.17	NS
N retained, %	45.4	49.3	45.0	44.9	2.52	NS
Protein deposition, g/d	221.4	258.1	214.0	225.3	13.5	NS

[1]Values are least-squares means (n=10 pens/treatment); DON, deoxynivalenol. Data were analyzed as a randomized complete block design. Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$.
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]SEM, Standard error of means
[7]N, nitrogen
[8]ATTD, Apparent total tract digestibility
[9]NS, Not significant ($P > 0.05$)
[10]Protein deposition = N retained × 6.25
[a b]Means without a common superscript within a row are significantly different ($P < 0.05$)

Table 12 Grower phase (d 0 – 42) serum chemistry panel for liver and kidney metabolites analysis[1]

Item	Day	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P*-value		
							Diet	Day	Diet × Day
Sodium, mM	0	144.6	144.5	142.9	142.8	0.77			
	14	145.8	145.9	145.4	142.9	0.77	NS[7]	0.001	0.015
	42	145.5	146.1	145.2	147.5	0.77			
Potassium, mM	0	6.52	6.33	6.29	6.29	0.44			
	14	5.96	6.21	5.46	5.46	0.44	NS	0.008	NS
	42	5.60	5.24	5.60	5.60	0.44			
Chloride, mM	0	96.6	96.4	96.0	95.3	0.63			
	14	95.8	97.2	96.6	96.2	0.63	NS	NS	NS
	42	96.3	96.5	96.3	97.3	0.63			
Bicarbonate, mM	0	19.4	21.0	20.6	20.8	0.87			
	14	18.6	21.1	21.4	20.8	0.87	NS	0.001	NS
	42	26.3	25.7	25.7	27.7	0.87			
Anion Gap, mM	0	35.4	33.4	32.6	33.6	1.23			
	14	37.5	33.8	32.8	32.1	1.23	NS	0.001	NS
	42	29.1	29.8	28.9	28.3	1.23			
Calcium, mM	0	2.88	2.85	2.78	2.82	0.05			
	14	2.95	3.04	2.88	2.78	0.05	NS	0.049	NS
	42	2.87	2.83	2.83	2.88	0.05			
Phosphorus, mM	0	3.32	3.25	3.19	3.26	0.08			
	14	3.38	3.19	3.10	3.13	0.08	NS	0.010	NS
	42	3.12	2.98	3.11	3.18	0.08			
Magnesium, mM	0	0.84	0.83	0.77	0.78	0.03			
	14	0.87	0.85	0.81	0.80	0.03	NS	0.040	NS
	42	0.86	0.86	0.86	0.87	0.03			

Table 12 (continued) Grower phase (d 0 – 42) serum chemistry panel for liver and kidney metabolites analysis[1]

Urea, mM	0	4.48	4.61	3.84	3.79	0.26		
	14	3.72	3.84	3.69	3.72	0.26	NS	0.001	NS
	42	4.99	5.43	4.72	4.80	0.26		
Creatinine, mM	0	69.0	73.6	68.6	71.9	3.55		
	14	82.5	85.7	82.6	86.5	3.55	NS	0.001	NS
	42	99.6	108.3	95.3	100.7	3.55		
Glucose, mM	0	6.57	6.23	6.41	6.36	0.00		
	14	6.92	6.14	5.97	5.97	0.00	NS	NS	NS
	42	5.82	6.10	5.85	6.20	0.00		
Total Bilirubin, mM	0	0.32	0.37	0.45	0.37	0.62		
	14	0.29	0.38	0.22	0.43	0.62	NS	NS	NS
	42	0.34	0.35	0.22	0.36	0.62		
Direct Bilirubin, mM	0	0.27	0.25	0.28	0.21	0.03		
	14	0.15	0.23	0.15	0.12	0.03	NS	0.001	NS
	42	0.20	0.16	0.11	0.18	0.03		
Indirect Bilirubin, mM	0	0.05	0.12	0.17	0.16	0.05		
	14	0.14	0.15	0.07	0.31	0.05	0.042	NS	NS
	42	0.14	0.22	0.11	0.18	0.05		
GGT[8], mM	0	32.4	30.1	32.6	35.9	2.78		
	14	34.2	32.6	34.0	34.8	2.78	NS	NS	NS
	42	34.2	33.7	33.9	38.4	2.78		
GLDH[9], mM	0	1.50	1.49	1.70	1.29	0.18		
	14	1.60	1.49	1.50	1.29	0.18	NS	0.007	NS
	42	1.33	1.10	0.90	1.11	0.18		
AST[10], mM	0	26.0	22.1	22.4	23.9	4.33		
	14	22.2	18.4	19.2	23.1	4.33	NS	NS	NS
	42	33.9	15.8	16.7	15.7	4.33		

Table 12 (continued) Grower phase (d 0 – 42) serum chemistry panel for liver and kidney metabolites analysis[1]

CK[11], mM	0	2192	1290	1159	1445	1229		
	14	4144	2723	2387	2360	1229	NS	0.001	NS
	42	8052	3622	8384	4200	1229		
Total Protein, mM	0	53.9	54.3	53.6	55.1	1.15		
	14	58.7	59.5	56.6	55.6	1.15	NS	0.001	0.049
	42	62.4	61.8	58.7	62.8	1.15		
Albumin, mM	0	13.1	13.6	13.6	15.4	0.97		
	14	15.9	15.7	15.7	16.7	0.97	NS	0.001	NS
	42	15.3	14.2	12.4	16.0	0.97		
Globulin, mM	0	13.1	13.6	13.6	15.4	0.97		
	14	15.9	15.7	15.7	16.7	0.97	NS	0.001	NS
	42	15.3	14.2	12.4	16.0	0.97		
Albumin:Globulin, mM	0	3.23	3.17	3.07	3.75	0.22		
	14	2.82	2.89	2.66	2.45	0.22	NS	0.001	NS
	42	3.23	3.45	3.75	3.02	0.22		

[1]Values are least-squares means (n=10/treatment); DON, deoxynivalenol. The data was analyzed above was analyzed as a repeated measure with 'day' as a repeated variable. Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$.
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of means.
[7]NS, Not significant ($P > 0.05$)
[8]GGT, Gamma-glutamyltransferase
[9]GLDH, Glutamate dehydrogenase
[10]AST, Aspartate aminotransferase
[11]CK, Creatine kinase

Table 13 Finisher phase (d 42 – 84) serum chemistry panel for liver and kidney metabolites analysis[1].

Item	Day	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P*-value		
							Diet	Day	Diet × Day
Sodium, mM	42	147.5	148.4	149.5	148.6	2.15			
	56	143.9	145.0	144.3	144.9	2.15	NS[7]	0.050	NS
	84	144.6	149.2	147.9	149.1	2.24			
Potassium, mM	42	5.80	5.46	5.66	5.16	0.19			
	56	5.25	5.54	5.35	5.32	0.19	NS	0.058	NS
	84	5.13	5.22	5.44	5.07	0.20			
Chloride, mM	42	98.1	97.8	99.2	98.4	1.47			
	56	95.0	95.9	94.9	95.4	1.47	NS	0.005	NS
	84	96.5	99.4	99.3	100.4	1.55			
Bicarbonate, mM	42	23.1	24.6	24.0	24.8	0.83			
	56	22.5	22.9	22.5	23.2	0.83	NS	0.001	NS
	84	26.4	27.6	28.5	28.7	0.87			
Anion Gap, mM	42	32.3	31.3	32.0	30.6	1.24			
	56	31.7	31.4	32.2	31.9	1.22	NS	0.001	NS
	84	27.1	27.0	25.8	25.1	1.27			
Calcium, mM	42	2.88	2.85	2.86	2.80	0.08			
	56	2.73	2.78	2.75	2.65	0.08	NS	NS	NS
	84	2.60	2.81	2.81	2.98	0.08			
Phosphorus, mM	42	3.15	3.06	3.07	3.06	0.08			
	56	2.95	2.93	2.84	2.82	0.08	0.022	0.001	0.25
	84	2.63	2.71	2.43	2.28	0.08			

Table 13 (continued) Finisher phase (d 42 – 84) serum chemistry panel for liver and kidney metabolites analysis[1].

Magnesium, mM	42	0.80	0.78	0.83	0.78	0.03		
	56	0.81	0.77	0.73	0.75	0.03	NS	0.001	NS
	84	0.87	0.91	0.87	0.88	0.03		
Urea, mM	42	5.96	5.80	6.12	6.10	0.33		
	56	5.56	5.65	5.37	5.49	0.33	NS	0.020	NS
	84	5.81	6.05	6.03	5.64	0.34		
Creatinine, mM	42	91.3	96.2	95.1	96.4	5.09		
	56	106.8	106.7	108.1	108.0	5.09	NS	0.001	NS
	84	126.5	135.9	133.3	133.3	5.27		
Glucose, mM	42	5.83	5.69	6.00	5.56	0.24		
	56	6.19	5.25	5.43	5.26	0.24	NS	0.007	NS
	84	5.34	5.25	5.49	4.96	0.26		
Total Bilirubin, mM	42	0.23	0.07	0.18	0.22	0.12		
	56	0.23	0.17	0.40	0.10	0.12	NS	NS	NS
	84	0.37	0.41	0.23	0.35	0.12		
Direct Bilirubin, mM	42	0.15	0.10	0.10	0.19	0.04		
	56	0.12	0.13	0.07	0.06	0.04	NS	0.003	NS
	84	0.18	0.26	0.16	0.19	0.04		
Indirect Bilirubin, mM	42	0.08	0.00	0.08	0.03	0.11		
	56	0.11	0.04	0.33	0.04	0.11	NS	NS	NS
	84	0.19	0.15	0.06	0.16	0.12		
GGT[8], mM	42	34.2	33.6	29.1	32.9	2.73		
	56	32.1	33.1	29.9	32.8	2.73	NS	0.040	NS
	84	35.5	36.6	31.6	36.5	2.83		

Table 13 (continued) Finisher phase (d 42 – 84) serum chemistry panel for liver and kidney metabolites analysis[1].

GLDH[9], mM	42	34.15	33.69	29.10	32.86	2.73		
	56	32.05	33.10	29.90	32.76	2.73	NS	0.040	NS
	84	35.49	36.60	31.57	36.51	2.83		
AST[10], mM	42	24.87	17.42	19.40	19.83	3.97		
	56	27.07	18.44	26.40	23.53	3.20	NS	NS	NS
	84	15.81	20.84	17.97	16.87	4.09		
CK[11], mM	42	3589	3593	3706	3619	914		
	56	4110	2803	4151	3144	914	NS	0.040	NS
	84	2409	2224	2808	2280	958		
Total Protein, mM	42	63.29	62.71	63.40	64.18	1.76		
	56	61.59	60.83	61.50	62.38	1.76	NS	NS	NS
	84	61.46	63.93	62.11	65.94	1.86		
Albumin, mM	42	48.96	48.27	47.60	47.90	1.15		
	56	47.66	46.46	46.50	46.70	1.14	NS	NS	NS
	84	47.48	48.66	47.30	47.85	1.19		
Globulin, mM	42	14.37	14.40	15.80	16.25	0.89		
	56	13.97	14.33	15.00	15.65	0.89	NS	NS	NS
	84	13.97	15.23	14.82	17.90	0.93		
Albumin:Globulin, mM	42	14.37	14.40	15.80	16.25	0.89		
	56	13.97	14.33	15.00	15.65	0.87	NS	NS	NS
	84	13.97	15.23	14.82	17.90	0.91		

[1]Values are least-squares means (n=10/treatment); DON, deoxynivalenol. The data was analyzed above was analyzed as a repeated measure with 'day' as a repeated variable. Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$.
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet

[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of means.
[7]NS, Not significant ($P > 0.05$)
[8]GGT, Gamma-glutamyltransferase
[9]GLDH, Glutamate dehydrogenase
[10]AST, Aspartate aminotransferase
[11]CK, Creatine kinase

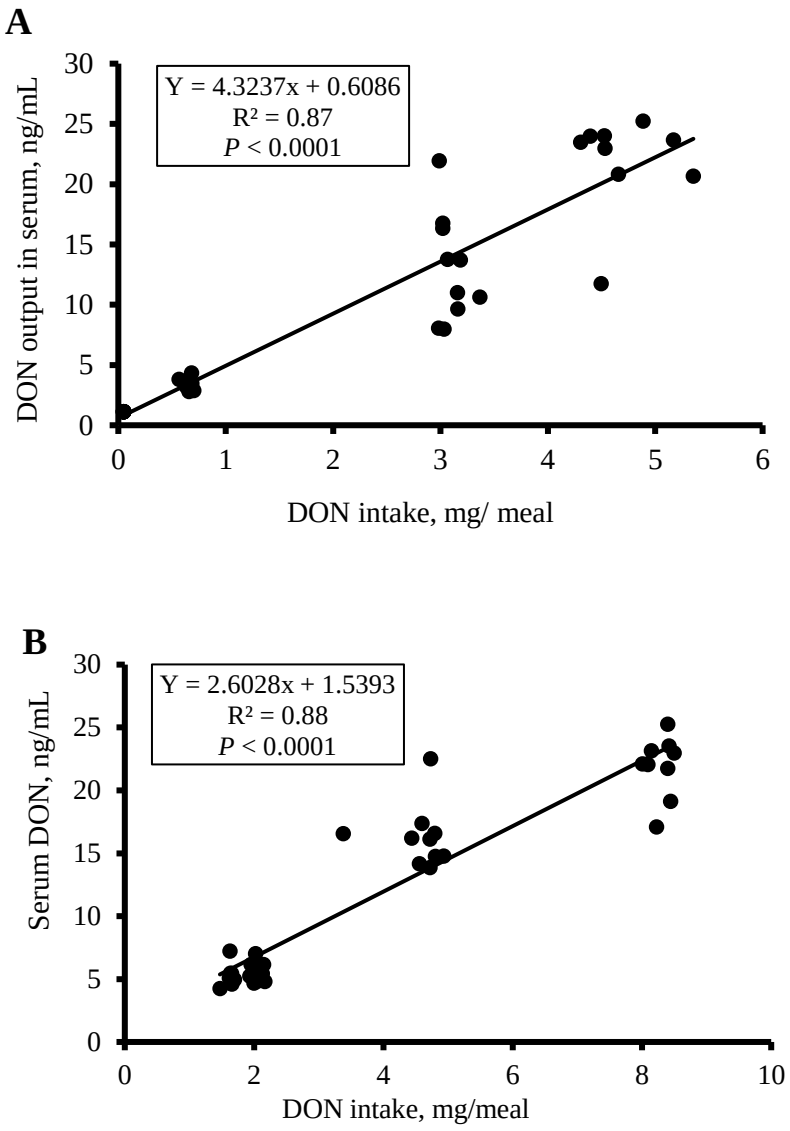

Figure 8 Regression analysis of the relationship between deoxynivalenol (DON) intake and DON output in serum (n=10 pigs/treatment). The serum samples were collected during the nitrogen balance period of experiment 3-4h after a single meal and analyzed for DON content. The figure A represents the grower phase and figure B represents the finisher phase of the experimental animals. Data are expressed as the DON intake (mg/meal) and the serum DON output (ng/mL) and the coefficient of determination (R^2) of the regression curve is 0.87 and 0.88 for figure A (grower phase) and figure B (finisher phase) respectively at $P < 0.0001$.

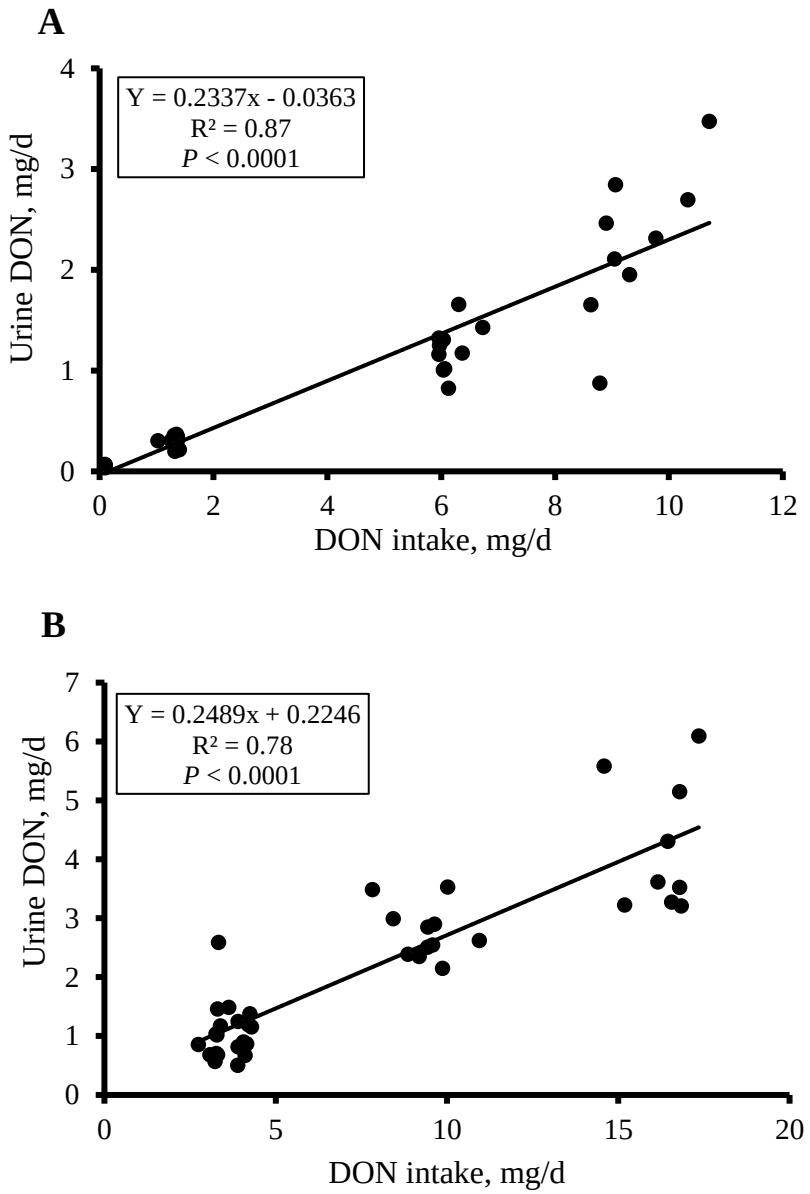

Figure 9 Regression analysis of the relationship between deoxynivalenol (DON) intake and DON output in urine (n=10 pigs/treatment). The urine samples were collected during the nitrogen balance period of the experiment over 24-h and analyzed for DON content. The figure A represents the grower phase and figure B represents the finisher phase of the experimental animals. Data are expressed as the DON intake per day (mg/d) and the urine DON output (mg/d) and the coefficient of determination (R^2) of the regression curve is 0.87 and 0.78 for figure A (grower phase) and figure B (finisher phase) respectively at $P < 0.0001$.

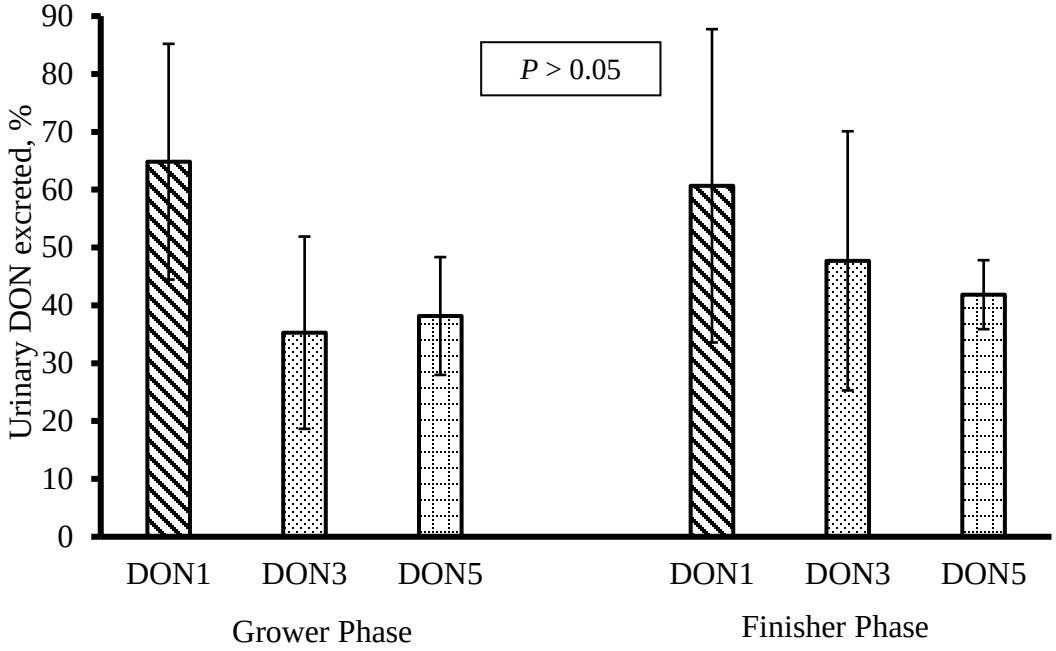

Figure 10 Deoxynivalenol (DON) recovery in urine expressed as a percentage of DON intake per pig per d for both the grower and finisher phases fed diets containing 1, 3, or 5 ppm DON (DON1, DON3, DON5, respectively. Bars represent urinary DON recovery shown as least square means ± SEM (n=10 pigs/treatment). Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$

4.3.6 Immune response measurement

Immune response data presented in Fig. 11 shows that there was a day effect ($P < 0.05$) on IgG titers in response to the Leptospirosis vaccine, however, there were no effects of diet or diet x day interaction ($P > 0.05$).

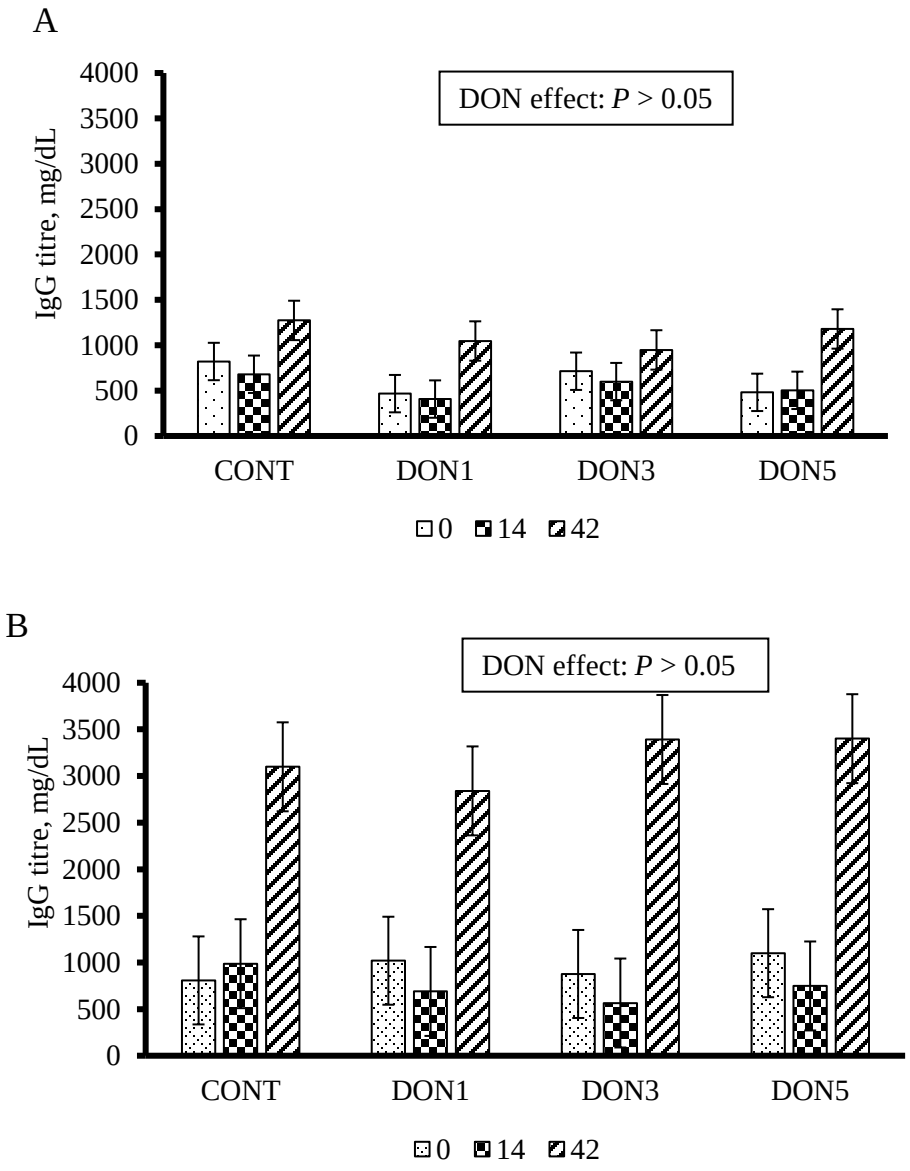

Figure 11 Serum IgG titers (mg/dL) in response to *Leptospira spp*. for grower phase (A) sampled on day 0, 14, and 42 and finisher phase (B) samples taken on day 42, 56, and 84 of the experiment representing d 0, 14, and 42 during the finisher phase respectively for pigs fed DON-contaminated diets (n=10 pens/ treatment).

Bars represent least-square means ± SEM (n=10 pigs/treatment). Differences between treatment means were determined using the Tukey-Kramer mean separation test with significance determined at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$

5.0 GENERAL DISCUSSION

Mycotoxin contamination in livestock diets is considered a global challenge. More so, the effect of DON contamination of cereal grains, which is increasing, has a significant impact on swine production. In general, pigs are the most affected livestock species by DON contamination as evidenced by physiological and growth performance responses reported in pigs fed DON-contaminated diets (Serviento et al., 2018). The reported effects of feeding pigs DON-contaminated diets include poor growth (Serviento et al., 2018; Girardet et al., 2011), impaired health (Pestka, 2010; Chaytor et al., 2011), and poor nutrient utilization (Santurio, 2000). These effects have been reported in pigs of all ages, however, there is some evidence that younger pigs may be more susceptible, even at lower doses (Alizadeh et al., 2015; Dersjant-Li et al., 2003; Chen et al., 2008; Savard et al., 2015). The majority of previous studies have focused on shorter exposure times (Accensi et al., 2006; Alizadeh et al., 2015; Dänicke et al., 2017) and assessed effects in younger pigs, therefore, this thesis examined the effects of feeding graded levels of DON to both grower-finisher (35 – 120 kg) and finisher (75 – 120 kg) pigs on growth performance, nutrient utilization, carcass characteristics, and overall health status during a long-term (42-d and 77-d, respectively) period. Although the CFIA has set the maximum allowable limit for DON in complete swine diets at 1 ppm, there is evidence indicating that pigs may have the potential to tolerate higher DON levels (Rotter et al., 1995; House et al., 2002; Dänicke et al., 2017; Serviento et al., 2018). Besides, some studies have suggested that pigs may be able to adapt to the effects of DON-contaminated diets during an extended exposure period (Rotter et al., 1994; Rotter et al., 1996; Serviento et al., 2018). The potential for recovery and adaptation may offer producers the opportunity for increased use of feedstuffs contaminated with DON in swine diets, provided economic impact is favorable. Therefore, the objective of this thesis was to assess the effects of feeding graded levels of DON on growth performance, nutrient utilization, health, and carcass characteristics of grower-finisher pigs. We also aimed to determine the difference in performance response between younger (grower pigs) and older pigs (finisher pigs) to DON contamination. Finally, given the difficulties with determining mycotoxin content in feeds and feedstuffs, we aimed to evaluate the effect of DON content in biological samples, such as urine and blood, and whether these could be used to determine actual DON exposure in pigs.

5.1 DON concentration in the experimental diets: analyzed vs. calculated

The experimental diets presented in this thesis were formulated to target dietary DON levels at 0, 1, 3, and 5 ppm. For the most part, the formulated and analyzed levels of the DON in the complete diets were similar. The major exception to this was the variation observed in DON level for the control diets used in the N-balance determination, which was analyzed to be 1.01 ppm for the grower period in experiment 1, and 1.56 ppm in the finisher period in both studies. This highlights the difficulty in analyzing actual DON content in feedstuffs and knowledge of final DON concentration in complete feeds, as the same ingredients were used for both the diets used in manufacturing the diets for growth performance and N-balance. Kong et al. (2016) reported DON levels ranging from 2.94 to 5.07 mg/kg in complete diets even though the same amount of DON-contaminated barley was used in the diet formulation. Similarly, Patience et al. (2014) reported that when two diets were formulated to have the same level of DON using the same amounts of DON-contaminated corn, the analyzed DON levels in the two diets were significantly different (0.25 mg/kg vs. 4.6 mg/kg). These studies further demonstrate the inconsistency in estimating DON contamination levels in complete diets.

Some reports suggest that the inaccuracies in the estimation of DON in complete diets could be due to the mechanisms by which *Fusarium* infects grain and proliferates in spots or localized portions of the grain batch during storage, leading to uneven distribution of *Fusarium* growth in samples (Swamy et al., 2002; Kong et al., 2015; 2016). Other factors that may influence the overall accuracy of testing for DON include sampling methods, sample size, sample preparation, including particle size, and methods of analyzing samples (Freese et al., 2000). For example, one study reports that when testing samples for DON in the whole grain, there is a greater variation (SD = 76) compared to samples tested after grinding (SD = 56; Champeil et al., 2004). This observation was confirmed by Sanders et al. (2013) who recorded very high levels of DON in wheat dust compared with whole wheat grains. The sample size also influences the accuracy of results, with a range of about 100 – 200 g and sampling from multiple locations being recommended as ideal (Freese et al., 2000). Results from our mycotoxin analysis of the experimental diets showed that other potentially harmful mycotoxins such as ZEA, FUM, and ergot alkaloids were significantly below harmful levels (CFIA, 2017), as such all effects and responses recorded will be assumed to be attributed solely to DON contamination. This is not to disregard the fact that mycotoxin co-contamination is widely researched and reported in the

literature, especially in grains naturally contaminated with *Fusarium* mycotoxins, since co-contamination can alter and or increase the severity of the response (D'Mello et al., 1999; Bracarense et al., 2012; Schatzmayr and Streit, 2013; Pinotti et al., 2016; Alassane-Kpembi et al., 2017). Results from the mycotoxin analysis in the experimental diets also suggest that swine diets in current commercial use may contain DON and potentially other mycotoxins.

5.2 Growth performance of pigs fed DON-contaminated diets

There is evidence that feeding DON-contaminated diets to pigs affects performance (Serviento et al., 2018), including average daily gain, feed intake, and feed efficiency. Factors that compromise feed intake will directly reduce nutrient intake, hence growth will be impaired (Pastorelli et al., 2012; Kong et al., 2016). Results presented in this thesis agrees with the CFIA recommended levels of 1 ppm in complete diets for pigs such that consistently and throughout both Expt. 1 and 2, there were no significant differences between pigs fed the CONT diets and pigs fed the DON1 diets. In the finisher experiment (Expt. 1), during the first week of the study, we observed a 34 % reduction in ADFI of the DON5-fed pigs compared to the CONT pigs, whereas pigs fed DON3 treatment showed less of an effect from DON with a 14% reduction in ADFI and pigs fed DON1 diets showed no effect due to DON. The reduction in respective ADFI across the treatments subsequently resulted in a 53% drop in ADG of the DON5-fed pigs with DON3-fed pigs showing a lower impact with a 27% drop in ADG. This observation is consistent with a previous report by Serviento et al. (2018) where pigs were fed with DON-contaminated diets over two periods with a DON-free treatment between the 2 periods and showed a 30 % reduction in ADFI and a subsequent 54 % reduction in ADG compared to the pigs fed no DON diets during the same period (d 0-7) of exposure in period 1. We further observed that the negative effects due to continuous exposure to DON-contaminated diets on ADFI and ADG were reduced over time where DON1 and DON3 treatment groups were statistically not different from the control group (CONT), as indicated by the lack of effect of dietary treatment on ADG and ADFI after 4 weeks of exposure. Overall, the ADG for DON3 and DON5 was lower when compared to DON1 and CONT. ADFI for DON5 pigs was lower than all other treatments and there was no effect of feeding DON on G:F. The observed results on ADG suggest that the initial exposure period (d 0-7) to DON elicited the greatest impact on ADG, followed by a steady decline in the severity of the effects of DON until there was no effect on performance, similar to previous reports (Li et al., 2011; Van Le

Thanh et al., 2015). This result provides evidence that pigs fed 5 ppm DON may adapt to the DON exposure, with recovery in performance after an initial negative response. The DON3 and DON1 treatments showed less of an effect and quicker adaptation, where DON1 pigs were largely unaffected by DON intake and responded similarly to the CONT pigs. Studies have suggested that nutrient, utilization, and protein synthesis may be compromised by DON intake in pigs (Chaytor et al., 2011), but no impact on feed efficiency was observed except during the first week in the DON5-fed pigs. This may suggest that in finisher pigs, the effects of DON intake on growth performance may be largely due to the effects of DON on feed intake. These observations agree with a meta-analysis by Pastorelli et al. (2012), which reported that 80% of the reduction in ADG observed with mycotoxin contamination in pigs could be related to impaired feed intake.

Growth performance results from Expt. 2 showed that ADFI was reduced by 11 – 12% in the DON5 and DON3 fed pigs compared to the CONT group with no effect on DON1 fed pigs during the d 0–7. The effect on ADFI resulted in a 22% and 17% drop in ADG for the DON3 and DON5 fed pigs, respectively. This observation confirms previous reports in terms of the initial response and effects of growing pigs fed DON-contaminated diets (Li et al., 2011; Van Le Thanh et al., 2015). Generally during the grower phase (d 0-42), on a week to week basis, there was no significant variation between treatments for ADFI except during d 0-7, and d 22-28. The ADG was therefore not affected beyond d 7 during the same phase (d 0–42), as was reported previously (House et al., 2002; Dänicke et al., 2004). During the finisher phase (d 43–77), there was a significant reduction (15%) in ADFI for DON5-fed pigs, whiles an 8% reduction was observed during d 43–49, and a 6% reduction in DON3 pigs from d 50 – 56. These results are unexpected as pigs had already been exposed to DON for an extended period and therefore the results may be attributed to the switch from the grower phase diet to the finisher phase diet. Again, individual animal variability or dose of DON contamination during that period may account for the observed response as previously argued (Nguyen-Ba et al., 2020). The reduced feed intake during the early periods of the finisher phase resulted in a 15% reduction in ADG for pig groups fed the DON5 treatment during the d 43–49 period only. There was, however, no further effect of treatment on ADG during the rest of the finisher phase (d 50–77). Overall, there was no effect on G:F throughout the entire experiment for both growth phases. The ADFI was more affected during the finisher phase (d 43-77), with no effect during the grower phase, whereas ADG was affected only during the grower phase (d 0-42). The difference in BW gained at the end of the grower phase was 3.6 kg

representing a 4% reduction in BW gain for DON5 pigs when compared to the pigs on the clean diets, while DON3 pigs were 3.9% lighter than the control group. At the end of the study, the overall reduction in BW gain in the DON3 and DON groups was 3.1% and 3.9% respectively but had no impact on carcass traits (House et al., 2002).

In the grower-finisher experiment (Expt. 2), it was evident that the initial exposure of grower pigs to the DON5 diets showed a less drastic effect compared to the finisher pigs, with a 10% vs 34% drop in ADFI, respectively, during the first 7 d. This trend was also observed during the same period for ADG, with grower pigs showing a 17% drop in ADG compared to a 52% reduction in ADG observed in finisher pigs. For DON3 pigs, on the other hand, ADFI and ADG were reduced by 14% and 27% during the first 7 d of exposure while in the grower-finisher study, there was a reduction of 12% and 22% for ADFI and ADG compared to the control group. Throughout the 2 studies, finisher pigs showed a gradual reduction in adverse effects whereas grower pigs seem to recover relatively quickly after the initial drastic reduction by DON on both feed intake and daily gain. There was a clear indication that the response for the grower pigs is not the same as was observed for the finisher pigs (Expt. 1) and by others (Flannery et al., 2011; Nguyen-Ba et al., 2020). However, at the end of the grower phase (d 0-42) for experiment 2, there was no effect of DON on ADFI but ADG had been reduced by only about 7% for pigs fed the highest DON (DON5) compared to pigs on clean diets (CONT). Comparing results in the two studies, finisher pigs showed more severe effects due to DON intake compared to grower pigs, suggesting that grower pigs may be less susceptible to DON exposure in diets.

It is thought that pigs have the potential to adapt to DON, and therefore a week by week regression analysis was used to establish the relationship between actual DON intake and the relative change in BW and ADG in both Expt. 1 and 2. In Expt. 1 (finisher study), pigs fed DON-contaminated diets show a consistent depression in BW gain and ADG, which show a gradual recovery over time. On the contrary, during Expt. 2 (grower-finisher), we observed a less drastic initial response and a greater degree of variability in response to DON intake. The longer exposure to DON during Expt. 2 (grower-finisher), resulted in an overall drop in ADFI and ADG of 5.7 % and 5.2 %, respectively, whereas in Expt. 1 (finisher) ADFI and ADG were reduced by 13% and 16% respectively when DON5 was compared to CONT. Also, this variability may due to variations of individual animal responses which may suggest that recovery from DON contamination may be

based on individual animal traits such as size and stage of development as well as resilience to DON (Nguyen-Ba et al., 2020).

In both Expt.1 and 2, DON1 fed pigs responded similarly to the CONT fed pigs, as such there were no significant differences observed for all performance parameters measured – BW gain, ADG, ADFI, and G:F. Again, comparing DON5 to the CONT group of pigs, there was only a 4% drop in final BW for Expt. 2 compared to the 7% for Expt. 1. The final BW of pigs on the DON3 diets were not different from DON5. When the final BW of DON3-fed pigs was compared for both experiments, there was only a reduction of 2% for Expt. 1 compared to 3% for Expt. 2. This effect of DON on final BW for DON5, when compared to CONT, was affected by the initial response (d 0-7) in ADG which was a 53% and 17% reduction for the finisher and grower pigs respectively, including a 34% and 10% lower ADFI for finisher and grower pigs respectively. Results from this study, therefore, suggest that younger animals may be less susceptible to DON intake and might take less time to recover as compared to older pigs (Serviento et al., 2018; Van Le Thanh et al., 2015; Reddy et al., 2018). The effect of DON on ADFI may be due to various mechanisms including palatability attributed to the growth of mold in the diets (Higgins and Brinkhaus, 1999; Serviento et al., 2018). Serviento et al. (2018) and Goyarts and Dänicke (2005), further reported that DON-fed to pigs had altered feeding behavior i.e., feeding frequency was reduced) implying that the pigs may not have sufficient feed intake to meet their daily nutrient requirements, reducing performance.

In effect practical approaches to the use of DON-contaminated ingredients in swine diets might be to evaluate the feasibility of feeding smaller frequent meals, however, this may impose challenges in the commercial setting. Nguyen-Ba et al. (2020) further suggested that there may be the potential for exploitation of breeding programs for the development of swine with low susceptibility to DON and other mycotoxins citing that, the phenotypic measurements of this trait are highly heritable. Also, the nutrient density of diets could be adjusted such that for the same amount of energy intake, there is more than the required amino acids and other nutrients as suggested by the NRC (2012). This may afford pigs fed high DON diets to have adequate nutrient intake even at a reduced feed intake such that reduction in BW gain will be minimized. As shown previously, pigs have the potential to recover from the anorexic effect of DON when subsequent or continuous exposures are introduced (Serviento et al., 2018). Strategies for dietary adjustment

of nutrients will have to consider this observation to meet targeted growth levels when feed intake is reduced.

5.3 Effect of DON on nutrient utilization and carcass characteristics

A nitrogen (N) balance experiment measures N retention with the assumption that the majority of this retention is as lean tissue gain (i.e., protein deposition) in growing animals (Wu, 2018). Nitrogen intake is important for lean gain and therefore, factors that affect N intake directly or N utilization affect protein deposition. There is evidence that suggests that DON intake might impact protein deposition in pigs (Swamy et al., 2002; Arthur and Herd, 2005). In mice, protein synthesis has been reported to be inhibited by DON intake (Azcona-Olivera et al.,1995). While we attempted to determine the impact of DON intake on N retention in the present studies, the lack of a true control diet due to the high levels of DON analyzed in the CONT diets, makes interpretation of the results difficult. In effect, the CONT group showed a more drastic response because DON levels in their diets during the growth performance were significantly lower compared to N-balance diets. Exposing pigs to DON at any stage in their growth has been shown to significantly affect performance within the first week (d 0-7) of the exposure by reducing voluntary feed intake (Serviento et al., 2018). However, when the issue of voluntary feed intake is eliminated by feeding pigs according to their body weight, as in an N-balance study, there is still an impact on the performance of pigs, suggesting that other factors, such as nutrient utilization, may be at play. Other factors that could be responsible include other physiological differences and potential effects on the microbiome of the pigs which together work for the efficient growth of the pig. These factors, however, were not addressed in this study.

During Expt. 1 the digestibility of N was observed to be increased with the intake of the DON-contaminated diets (DON1, DON3, and DON5) compared to the control group (CONT) by as much as 8 – 10% which is in agreement with Goyarts and Dänicke, (2005) where DON-contaminated diets led to a significant increase in protein digestibility up to 6%. This was most likely because the CONT group were being exposed to greater levels of DON compared to what they were exposed to during the performance period. Dänicke et al. (2004) and Kong et al. (2016) reported that there were no effects of DON on N digestibility, which suggests that the response to DON in diets of finishing pigs can be variable depending on factors such as the individual animal variability, and the duration of exposure. In our current study, the CONT-fed pigs which were fed

diets containing 0.11 ppm DON during the growth performance period were effectively exposed to diets containing 1.56 ppm DON during the nitrogen balance period, which when inferring from the performance results, drastically had a negative effect on the pigs during the balance study. This is evident in the overall N-retention of the CONT group, which was not different from the high DON diets (DON3 and DON5). In principle and based on the results, the pigs fed the DON1 diets represent more of a control group which was quite evident in the overall high retention of N (65%) compared to the DON3, DON5 and, CONT pigs. The CONT group, however, can be argued to represent the effect of DON on nutrient utilization when DON is initially exposed to growing pigs. Though digestibility was low, there was also a significant amount of N wasted through the output of N in both urine and feces. Though there is no carcass data to support the overall translation of this result into carcass traits, the lack of effect of DON feed efficiency (G:F) during the performance period suggests that the initial exposure of pigs to DON reduces ADFI and ADG, and overall BW, however, as these growing pigs continue to feed on this same DON-contaminated diet, there is some recovery (ADG and ADFI) leading to an overall reduced effect. It is possible that the recovery time is insufficient to recover BW. The overall results, therefore, suggest that DON may have detrimental effects on nutrient utilization.

During the growth phase of Expt. 2, CONT-fed pigs showed a significantly higher PD of 227 g/d compared to pigs fed DON3 and DON5 diets (193 and 174 g/d, respectively) but was not different from DON1 pigs (200 g/d). Though DON1 showed significantly lower digestibility of N compared to CONT and CONT was not different from both DON3 and DON5 pigs. During the finisher phase, on the other hand, CONT treated pigs had a higher digestibility of N compared to all other treatments (DON1, DON3, and DON5), however, there was no difference in final protein retention hence PD, which is supported by the growth performance data where G:F was not affected by the treatment of DON-contaminated diets for the overall exposure period. This agrees with Van Le Thanh et al. (2015) when diets containing 4.6 ppm DON were fed to 6 kg piglets for 14 days and earlier results from House et al. (1986). The results from Expt. 2 suggests that, though in the short-term there could be depressed nutrient utilization as seen in the grower phase, extended exposure to DON affords growing pigs the opportunity to recover from any adverse effects of DON on nutrient use. Further, we observed that in the growth performance experiments, when the feed efficiency of pigs fed DON-contaminated diets was compared to pigs on control diets, there was no significant effect of DON, suggesting that the pigs were able to efficiently utilize nutrients

for growth. This observation is supported by the measured blood urea nitrogen data, which was not affected by dietary treatment and not different over time, and there was no treatment by day interaction, suggesting that the efficiency of uptake and utilization of N was not affected. In a study by Van Le Thanh et al. (2015), there was no impact of feeding DON to pigs on their N retention as was similar to results observed in the finisher phase of Expt. 2. On the contrary, the significant adverse effects of DON on N retention during experiment 1 may be due to the short exposure period (42 days) and specifically to the finisher experiment, may arise from the excessively high differences in DON levels between the diets used in the performance period (0.11 ppm) vs. diet used in the N-balance period (1.56 ppm). The gradual recovery rate of pigs from the drastic reduction in ADFI, which leads to a drop in ADG, versus the sustained negative effect on ADFI and the potential for DON to limit feed efficiency may have been rapid enough such that the negative effects of DON are managed or reduced as the pigs grow to adapt to the contaminated feed. Overall, the poor growth performance exhibited by pigs fed DON-contaminated diets can be attributed mainly to the limited feed intake rather than impaired feed utilization, especially at relatively low concentrations of 1 to 5 ppm used in this thesis as was also reported by Pastorelli et al. (2012).

When CONT, DON3, and DON5 diets were fed to pigs in Expt. 1, it was apparent that the DON1-fed pigs responded as a control group and showed the higher PD compared to all other treatments but specifically was 36, 42, and 29% higher than CONT, DON3, and DON5-fed pigs, respectively. Interestingly, when there was no DON contamination in the diets of the CONT-fed pigs during the grower phase of Expt. 2, CONT-fed pigs showed only a 15 – 24% higher PD compared to DON3 and DON5 pigs, which is proportionally lower than the PD compared to Expt. 1. Overall, however, when the finishing pigs in Expt. 2 were fed DON-contaminated diets there was no significant effect of the treatment suggesting a level of recovery and adaptation by the end of the Expt. 2. This further supports the results from the growth performance data, suggesting that younger pigs are less susceptible to DON-contaminated diets up to 5 ppm compared to older finisher pigs, and the continuous feeding of DON-contaminated diets may alleviate the overall negative effects of nutrient utilization. In the current study, the N-balance was carried out at the end of the exposure periods of the studies, which are after any potential adaptation, hence a lack of response may have been due to timing and not just the effect of DON. However, as indicated earlier the unexpectedly high DON content of the CONT diets used in Expt. 1 and the finisher

phase of Expt. 2 revealed the potential response to DON had the nitrogen balance been carried out by the end of the first week of exposure. The results are also supported by the response observed in the lack of effects of DON on feed efficiency (G:F) and urea levels measured in serum samples. Similar to reports by Bergsjø et al. (1993), House et al. (2002), and Serviento et al. (2018), our results showed that there was no impact of feeding DON-contaminated diets on carcass traits, suggesting that feed intake and nutrient utilization had largely recovered, despite a lack of recovery in body weight.

5.4 DON effect on health and blood metabolites

Liver and kidney metabolite analyses are key in identifying changes in the metabolism and health status of pigs following a period of ingestion of any toxin (Renner et al., 2017). Since DON is reported to be metabolized mainly in the intestine, liver, and kidney (Schwartz-Zimmermann et al., 2015), we evaluated kidney and liver metabolite changes in DON-fed pigs over time and observed no significant changes overall compared to pigs fed a control diet. The effects of DON contamination, or the lack of it thereof, were consistent with results from Rotter et al. (1995), Accensi et al. (2006)**,** and Sayyari et al. (2018)**.** Rotter et al. (1995) in a 42-d study, fed diets up to 4 mg/kg DON and reported that DON reduced the synthesis of protein in the liver, however, the pigs recovered to normal feed intake. Similarly, weanling pigs fed graded DON levels (0, 280, 560, or 840 µg/kg) show no effect of DON intake on hematological, biochemical changes in the blood (Accensi et al., 2006). Increasing dietary DON levels had no effects on selected liver and kidney metabolites, some blood proteins, glucose, and electrolytes. Blood urea measurements, which are important to understand renal function, protein utilization, and efficiency (Kong et al., 2015), were not affected by the increasing levels of DON in the experimental diets. A study by Accensi et al. (2006) observed similar results, though their study used piglets fed up to 840 µg/kg DON in contaminated diets over a 28-d. Further, Chaytor et al. (2011) also saw no effect of DON when it was in combination with AF on blood urea nitrogen in their study where they used 60 gilts (13.9 ± 0.2 kg of BW) for 33 d. These results were also in agreement with Kong et al. (2015).

There is some evidence that when DON is fed to growing pigs there is no immune stimulation in response to the mycotoxin (Bergsjø et al., 1993; Accensi et al., 2006; Weaver et al., 2013; Kong et al., 2016) but some other studies have shown otherwise (Swamy et al., 2002; Tiemann and Dänicke, 2007; Maresca, 2013). However, contrary to Chaytor et al. (2011) and

Reddy et al. (2018), during Expt. 2 no effect was seen when a humoral immune response was induced with the Leptospira vaccine since the IgG titers were not significantly different across all treatments. In a study by Øvernes et al. (1997), 5 different antigens namely human serum albumin (HSA), sheep red blood cells (SRBC), paratuberculosis vaccine (MPT), Tetanus Toxoid (TT), and Diphtheria Toxoid (DT) were used to try to elicit an immune response in young pigs fed DON-contaminated diets, HSA showed a primary immune response after 6-wk. TT was also observed to elicit a secondary immune response after 9-wk while the other antigens caused no immune response. According to Cano et al. (2013), there is evidence from work done in vitro that DON may increase susceptibility to diseases in pigs. This increased susceptibility of pigs to diseases was suggested to be due to not just DON but also its interaction with other mycotoxins such as ZEA and the nutritional effects caused by the DON-induced reduction in feed intake (Pestka et al., 1987). The lack of immune response also suggests that when DON limits feed intake, the nutrients pigs acquire are mostly used for growth and not for use by the immune system (Serviento et al., 2018). Results from this thesis agree with Kong et al. (2016) who concluded that there was no evidence of immune stimulation with DON intake. Overall, it appears that the intake of DON up to 5 ppm has little or no effect on animal health.

5.5 DON in urine and serum samples

Post-prandial plasma DON levels have been reported to reach a peak within 3-4 h of a meal, with DON concentration dropping over time to very low concentrations (Nagl et al., 2014; Paulick et al., 2018). Based on this, we measured DON levels in blood plasma in response to a meal to determine if this can be used as an indicator of DON exposure. Similar to Dänicke et al. (2004), we observed a dose-dependent increase in serum DON, indicating that the more DON is consumed, the more DON will be present in the blood. Serum DON levels, therefore, may be used as an indicator to predict DON intake. As indicated earlier, there were significant levels of detectable DON in the control (CONT) diets used in the finisher N-balance diets. This DON contamination was confirmed by significant levels of DON recovered from serum samples of pigs fed the CONT diets. Previous studies by Nagl et al. (2012) and Schwartz-Zimmermann et al. (2014; 2017) have indicated the main excretion route of DON is urine, hence, we also wanted to determine if urine can be used to determine DON exposure. In the present studies, urine samples collected during the N-balance and were analyzed for the presence of DON also showed similar correlating

patterns as were observed in the serum. There was generally a positive correlation of DON in urine samples to DON in the diet. Again, pigs fed the finisher CONT diets, which on the analysis contained significant levels of DON, were also observed to have DON in urine, supporting the analytical evidence of potential contamination of the diets. Results from this thesis agree with similar studies and further confirms that the main elimination route for DON is through urine (Dänicke et al., 2004; Nagl et al., 2014). Urinary excretion of DON accounts for more than 60% of all DON excreted in pigs, whereas fecal excretion accounts for trace amounts (Nagl et al., 2014). There was a dose-dependent increase in DON recovered from urine and serum samples (Schwartz-Zimmermann et al., 2017). Serum and urine can be therefore considered bio-assays to test DON exposure since DON excretion is time-dependent and occurs rather rapidly, which leads to reduction and recovery in feed intake levels as seen in our results and that of Flannery et al. (2012).

5.6 Summary and implications

This thesis set out to understand the effects of feeding graded levels of DON-contaminated diets to finishing and grower-finisher pigs over a longer-term compared to other studies. Indeed, DON negatively affected the ADG, ADFI, and the final BW of pigs, however, while ADG and ADFI recovered after 4 weeks during Expt. 1 and 7 - 8 weeks during the Expt. 2 final BW did not recover. There was also no indication of negative effects of DON intake on organ function or health of animals. Overall, our results show that the effects of DON on growth performance is mainly due to poor feed intake. Though results from Expt. 1 and 2 are not statistically comparable, it appears that younger pigs were less affected by DON intake, this was evident in the final BW weight difference between pigs fed DON5 and pigs fed the CONT diet. While DON intake affected growth and feed intake, it did not appear to influence feed efficiency (G:F) and there were no effects on carcass characteristics, suggesting that this feed intake effect may be managed or mitigated by the alteration of the nutrient content. Another practice that may potentially be applied and be beneficial to hog producers could involve altering feed regimes such that DON is introduced at specific periods where negative effects of performance will be reduced, such as in the grower period. Indeed, a cost and benefit analysis or the use of an economic model may be necessary to shed more light on the economic impact of the use of DON-contaminated diets against a more traditional feeding system where clean uncontaminated diets are fed to pigs. A modified feeding program may include DON in diets fed to pigs but will require increased days to market to maintain

the same final BW, as was observed by House et al. (2002). Analysis of DON content in the test diets of the current thesis demonstrates the difficulty in accurately and consistently determining the content of mycotoxins in feeds and feedstuffs. In this study, we demonstrate a strong positive correlation between DON intake to DON concentration in biological samples, specifically urine and serum. Since DON testing can be challenging, costly, and time-consuming in feeds and feedstuffs, analysis of biological samples to determine actual DON intake/exposure in pigs may be possible.

6.0 CONCLUSION

The studies presented in this thesis provide evidence that DON intake in grower and grower-finisher pigs have a negative impact on growth and feed intake, but little effect on nutrient utilization, health, and carcass characteristics. Also, there is evidence that pigs recover from DON intake after a period and the negative effects of DON are greater in finisher vs. grower pigs. The information in this thesis will allow for hog producers to develop strategies to minimize the effects of DON contamination in the feed while maintaining animal performance and health and production profitability. The results from this thesis will also support grain producers such that there may be an auxiliary market for Fusarium damaged kernels or grains.

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